UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
RESEARCH TRIANGLE PARK, NC 27711

February 1, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

Dr. Curtis Klaassen
Chair
External Peer Review Panel for *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information*
University of Kansas Medical Center
2018 Breidenthal Building
3901 Rainbow Boulevard
Kansas City, KS 66160

Dear Dr. Klaassen:

Please find enclosed a set of new analyses based on data that were not provided in sufficient time to include in the December 31, 1998 external review draft of the document *Perchlorate Environmental Contamination: Toxicology Review and Risk Characterization Based on Emerging Information.* These data represent important information that is being made available as part of completing the original set of studies in the testing strategy. We will present brief summaries of these data at the peer review meeting as falling into one of three different categories as follows:

1.	*Completed EPA analysis:*	EPA has finalized its analyses utilizing final audited data from a particular study.
2.	*Preliminary EPA analysis:*	EPA has either analyzed audited data for individual parameters but the final report audit is not completed, or the analyses EPA performed may not be complete.
3.	*Pending data:*	These are studies that are in the pipeline. Due dates and thoughts on how these data inform the current effort will be presented.

It has always been the intention of the National Center for Environmental Assessment (NCEA), lead for EPA in development of the assessment document, that this external peer review represent one piece of an iterative process. Once these preliminary analyses are completed and when the pending data are available as completed final reports, the document will be revised and undergo additional rigorous internal and external review. Recommendations made at this juncture on the existing document and the proposed model approach will be incorporated as well, so that while these data are being provided now in addition to those reviewed in the document, any extent to which the peer review panel would care to comment on them is welcome and would greatly enhance the next phase of the assessment effort.

It is NCEA's understanding that there is to be a package mailed to peer reviewers in early February, and another directly to San Bernardino on February 8th. Table 1 shows what is enclosed in this package from the ERD assessment team with an indication of who on the panel these new data should be brought to attention. Table 2 provides what is expected for the February 8th package.

The NCEA risk assessment team is looking forward to a stimulating and valuable peer review of these data and their anticipated interpretation / integration into the assessment effort. If there are any questions or if I can be of any additional assistance, please do not hesitate to contact me at 919.541.4847 (voice), 919.541.1818 (FAX) or E-mail (jarabek.annie@epa.gov).

Sincerely,

Annie M. Jarabek
EPA Perchlorate Assessment Team Leader and
Interagency Perchlorate Steering Committee
(IPSC) Executive Committee (NCEA)

Enclosures

cc: **w/o enclosures**

William Farland, NCEA IO
Lt. Col. Dan Rogers, IPSC Executive Committee (USAF)
Peter Grevatt, IPSC Executive Committee (OSWER)
Kevin Mayer, IPSC Executive Committee (Region 9)
Mike Osinski, IPSC Executive Committee (OW)

Table 1. Data Analyses Provided in February 1, 1999 Package

Data description	Status of EPA Analysis	Attention Panel Member(s)
1. Final genetox assays a) Repeat of Salmonella battery plus 2 additional strains by NTP b) Repeat of mouse micronuclei assay by NTP c) Repeat of mouse lymphoma by BioReliance	Final — Memos and revised text to document provided.	David Brusick
2. Brain histopathology at the 3 mg/kg-day dose from the Argus (1998a) neurodevelopmental study	Preliminary pending recommendations at peer review.	Tom Zoeller
3. Nonparametric Reanalysis of thyroid histopathology in pups on PND5 from the Argus (1998a) neurodevelopmental study	Preliminary — Provided in response to request by Joe Haseman to correct some data entries and to extend analysis with more exact procedures	Joe Haseman Susan Porterfield Tom Zoeller
4. Hormone data for F0 and F1 generation in 2-generation reproductive study (Argus, 1998b).	Preliminary — These particular data are audited but the overall final report and data have not been audited nor released. Analysis represents alternative approach suggested by Joe Haseman.	Tom Zoeller Joe Haseman Susan Porterfield
5. Reproductive parameters (sperm morphology and estrous cyclicity) from F1 generation in 2-generation reproductive study (Argus, 1998b).	Preliminary — These particular data are audited but the overall final report and data have not been audited nor released.	Rochelle Tyl
6. Sheep red blood cell (SRBC) assays from 90-day experiments in immunotoxicity studies	Preliminary — Data audited but final report not released.	Kimber White
7. Thyroid histopathology in mice from immunotoxicity studies	Preliminary — Data are audited but additional dose levels required for EPA to evaluate dose response	Tom Zoeller Susan Porterfield

Table 2. Data Analyses To Be Provided in February 8, 1999 Package

Data description	Status of EPA Analysis	Attention Panel Member(s)
1. Occupational cross-sectional study of workers exposed via inhalation and an epidemiological study	Preliminary — Manuscripts submitted as accepted on 1/22/99. EPA analysis not complete.	Susan Porterfield Tom Zoeller Charles Emerson
2. Sheep red blood cell (SRBC) from 14-day experiment (repeat) in immunotoxicity studies	Preliminary — Data audited but final report not released.	Kimber White
3. 14-day repeated dose pharmacokinetic study	Preliminary — Data are part of PBPK model development for interspecies extrapolation and completion of mode-of-action motivated model	Mel Andersen
4. Correlations between percent of iodide uptake inhibition and hormone perturbations using single dose and repeated 14-day dose PK studies	Preliminary — Data are part of PBPK model development for interspecies extrapolation and completion of mode-of-action motivated model	Mel Andersen Tom Zoeller

February 1, 1999 EPA Assessment Submission

Attachment #1
Final Genetox Review

A. **Final NTP Salmonella battery**
B. **Repeat of Mouse Micronuclei assay by NTP**
C. **Review of A and B by EPA (Dellarco memo)**
D. **Repeat of Mouse Lymphoma by BioReliance**
E. **Review of D by EPA (Moore memo)**
F. **Revised section of document**

ATTENTION PANEL MEMBER(S):

DAVID BRUSICK

January 28, 1999

NOTE TO: Annie Jarabek
FROM: Vicki Dellarco
RE: Review of the NTP Mutagenicity Studies on Ammonium Perchlorate

I have reviewed both the Ames assay and the mouse bone marrow micronucleus assay on ammonium perchlorate conducted under the auspices of the National Toxicology Program. Negative results were found in both assays. I find the protocols and the results from these tests to be acceptable. Furthermore, these recent studies confirm and reinforce the negative findings reported by another laboratory from these assays. I will revise the assessment document on perchlorate accordingly to reflect these new and important findings.

(NTP, 1999a)

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

Memorandum

Date: January 11, 1999

From: Errol Zeiger, Environmental Toxicology Program, NIEHS

Subject: Ammonium Perchlorate MN Summary Test Results

To: Annie Jarabek, National Center for Environmental Assessment, EPA

Male B6C3F1 mice were treated i.p with 125, 250, 500, 1000, 1500, and 2000 mg/kg ammonium perchlorate in buffered saline, plus solvent and positive (cyclophosphamide) controls. Five mice per group were injected daily for 3 consecutive days, and were sacrificed 24 hrs after the last injection. Their femoral bone marrow was removed and the polychromatic erythrocytes (PCE) scored for micronuclei (MN). All testing and scoring were done under code.

All animals in the 1500 and 2000 mg/kg groups died after the first i.p. injection, and 4/5 animals in the 1000 mg/kg group died after the second i.p. injection; the fifth animal was sacrificed and not scored for MN. All animals in the 125, 250, and 500 mg/kg groups survived the treatment; 2000 PCE's were scored per animal for MN.

The test data were analyzed statistically and have been entered into the NTP genetic toxicity database. No increases in MN-PCE were seen at any of the test doses, and the trend test was not positive. The positive control yielded a significant increase. No bone marrow toxicity was seen, as indicated by the percent PCE. The following table summarizes the results of the test.

mg/kg	mean MN cells/ 1000 PCE ± S.E.M.	pairwise p*	%PCE
0	3.00 ± 0.57		46.6
125	3.10 ± 0.40	0.4490	51.7
250	3.20 ± 0.56	0.3996	55.6
500	2.10 ± 0.29	0.8956	49.2
pos**			
15	19.60 ± 2.03	0.0000	56.5

trend test p = 0.903

* p value for pairwise comparison against the solvent (0 dose) control
** positive control, cyclophosphamide

The results of this study are consistent with those reported in the Perchlorate Study Group report (Study No. 6100-001). In that study, which used gavage administration, the highest dose that could be scored was 1000 mg/kg.

(NTP, 1999b)

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

Memorandum

Date: January 13, 1999
From: Errol Zeiger, Environmental Toxicology Program, NIEHS
Subject: Ammonium Perchlorate Salmonella Summary Test Results
To: Annie Jarabek, National Center for Environmental Assessment, EPA

The results of the NTP's Salmonella mutagenicity testing of Ammonium perchlorate are attached. The values presented are the means and standard errors of the mean, of triplicate plates.

The chemical was dissolved in water and tested using the preincubation procedure at doses from 100 to 10,000 µg/plate, without metabolic activation (NA), and using S-9 liver homogenates from Aroclor induced hamster (HLI) and rats (RLI). Two different concentrations of S-9 were used, 10% and 30%. The tests without metabolic activation (NA) were performed twice. Salmonella tester strains TA102, TA104, TA100, TA1535, TA97, and TA98 were used. "Pos" is the positive control.

Ammonium perchlorate was not toxic or mutagenic under the conditions of this test.

Although there were a number of differences between the NTP protocol and that used by the Perchlorate Study Group report (Study No. 6100-001), the conclusions of both tests are the same.

AMMONIUM PERCHLORATE

(LAB: SRI SOLVENT: H2O PROTOCOL: PREINC)

TA102

DOSE	NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	163	12.1	205	13.7	312	14.8	269	10.5	274	29.3	281	8.4
100.000	182	8.2	207	25.0	319	17.1	270	21.1	302	9.5	275	11.0
333.000	174	4.5	220	14.2	316	10.5	257	16.5	306	14.7	262	5.8
1000.000	161	3.3	232	7.8	287	25.2	265	15.3	296	9.0	276	10.4
3333.000	182	6.0	216	9.5	291	10.0	240	18.7	271	7.3	265	23.7
10000.000	176	10.2	190	31.2	317	7.1	256	16.3	280	24.8	270	4.1
POS	751	27.7	739	17.1	1182	17.9	1049	44.5	1043	52.0	942	18.1

TA104

DOSE	NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	247	12.7	317	20.7	436	12.4	334	15.7	422	23.6	334	17.3
100.000	280	9.7	341	17.6	439	18.7	344	21.5	404	16.7	310	11.3
333.000	254	26.3	318	18.8	374	60.4	373	8.7	426	8.2	344	17.6
1000.000	250	17.7	326	7.2	426	15.1	385	9.3	451	13.2	350	21.6
3333.000	272	15.1	338	19.3	424	12.7	351	13.7	413	18.6	344	27.6
10000.000	254	12.5	341	17.8	442	15.9	341	19.0	450	9.8	331	12.3
POS	847	25.7	843	28.0	1200	25.9	1260	12.5	962	18.6	1225	33.9

DOSE	TA100 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	155	5.2	128	2.2	125	13.7	173	4.6	126	7.3	147	3.8
100.000	152	3.2	121	6.5	128	0.6	161	10.0	131	3.5	161	3.8
333.000	155	3.5	124	4.0	132	4.9	155	13.8	122	3.3	151	8.1
1000.000	163	4.7	128	13.8	133	4.5	164	3.0	133	6.2	148	6.0
3333.000	147	7.2	132	4.8	121	2.6	172	8.6	135	11.9	159	2.6
10000.000	157	14.1	126	6.1	119	4.9	170	5.5	126	2.9	146	4.9
POS	928	7.2	937	18.8	629	9.2	722	12.4	540	14.8	657	20.5

DOSE	TA1535 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	12	2.2	9	1.8	16	3.2	9	1.8	15	1.5	11	1.5
100.000	13	0.6	9	1.8	13	3.5	10	0.7	16	0.9	15	2.7
333.000	10	1.3	14	1.9	11	0.9	14	3.2	9	2.1	12	0.3
1000.000	10	1.2	10	1.5	13	0.6	12	0.9	11	0.7	11	0.0
3333.000	13	3.3	12	1.2	10	1.5	12	0.9	12	1.7	13	1.9
10000.000	10	0.6	10	1.9	12	1.9	9	0.9	10	1.7	8	0.0
POS	835	18.2	856	11.6	152	8.7	131	8.4	137	8.7	110	6.7

DOSE	TA97 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	121	3.7	135	16.4	178	12.9	168	14.6	157	13.7	158	14.9
100.000	130	9.3	141	9.1	182	7.0	183	3.4	167	4.9	179	6.2
333.000	131	16.8	132	7.9	170	4.0	172	7.0	153	10.5	174	14.5
1000.000	140	7.4	161	3.7	162	6.4	191	3.1	149	8.1	168	5.8
3333.000	134	5.5	164	8.5	153	12.7	192	0.9	154	16.5	173	13.8
10000.000	124	4.0	122	6.3	177	12.4	131	9.9	143	11.3	167	5.2
POS	508	20.7	553	21.5	513	183.0	592	13.0	656	10.0	517	8.2

DOSE	TA98 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	22	4.1	24	3.0	25	3.8	17	1.8	27	3.8	24	1.3
100.000	17	1.7	29	5.9	35	3.3	22	2.1	32	3.2	23	2.4
333.000	17	0.9	21	3.8	26	2.8	20	2.5	29	0.9	24	3.0
1000.000	23	2.3	24	0.3	23	1.2	21	4.6	22	0.7	21	2.0
3333.000	18	3.5	21	1.5	30	2.0	17	3.8	35	3.8	20	3.1
10000.000	18	4.4	26	3.3	27	4.8	20	4.4	29	2.6	19	0.3
POS	355	17.6	362	7.7	543	12.9	545	16.9	466	18.2	536	45.1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK
NORTH CAROLINA 27711

MEMORANDUM

DATE: January 29, 1999

SUBJECT: Analysis of Perchlorate

FROM: Martha M. Moore, Chief (MD-68) Martha Moore
Genetic & Cellular Toxicology Branch

TO: Vicki Dellarco, (MD7509C)
Office of Pesticides Programs
Annie Jarabek, (MD-52)
Toxocologist

I have reviewed the mouse lymphoma data generated in the repeat analysis of perchlorate and based on this information, I am confident that the data are sufficient to determine the chemical to be nonmutagenic both with and without S9 activation. While I am a little concerned that the background mutant frequency is too low, particularly in the without S9 experiment, this data set looks overall to be very good. It is internally very consistent. The problems that were observed in the data generated by the first laboratory are not present in the data from this laboratory. The issue of low background mutant frequency relates to whether the laboratory is adequately quantitating all of the mutants. I think that the mutant colony sizing curves that are included in the data provides sufficient evidence that the laboratory is quantitating mutants properly.

BIORELIANCE℠
Formerly Microbiological Associates

BIORELIANCE CORPORATION
14920 BROSCHART ROAD
ROCKVILLE, MARYLAND 20850-3349 USA
PHONE: 301.738.1000 • FAX: 301.738.1036

January 27, 1999

Mr. Michael F. Girard
Perchlorate Study Group Representative
Highway 50 and Aerojet Road
Building 20019/Department 0330
Rancho Cordova, CA 95813-6000

Dear Mr. Girard:

Enclosed please find the original of the final report for the BioReliance study G98BA06.702, *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay), which was performed using your test article: Ammonium perchlorate. Also enclosed is the Response to Audit Comments.

Should you require additional information or have questions, please call Dr. Richard San at (301) 738-1000, extension 2222.

Sincerely,

Diane Gray
Secretary
Toxicology Testing Services

Enclosures

cc: Michael L. Dourson, Ph.D., DABT
Toxicology Excellence for Risk Assessment
4303 Hamilton Avenue
Cincinnati, OH 45223

Annie Jarabek (phone: 919-541-4847)
USEPA/NCEA
Progress Center
Catawba Building
3200 Highway 54
Research Triangle Park, NC 27709

R. San
P. Smith
Study file

Response to Audit Comments

Test Article ID: Ammonium perchlorate
MA Study No.: G98BA06.702
Report Type: Draft to Final

All changes requested by the Sponsor have been incorporated into the final report.

RS 1/27/99

FINAL REPORT

Study Title

In Vitro Mammalian Cell Gene Mutation Test
(L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

Test Article

Ammonium perchlorate

Authors

Richard H. C. San, Ph.D.
Jane J. Clarke, B.A.

Study Completion Date

January 27, 1999

Performing Laboratory

BioReliance
9630 Medical Center Drive
Rockville, MD 20850

Laboratory Study Number

G98BA06.702

Sponsor

Perchlorate Study Group
Highway 50 and Aerojet Road
Building 20019/Department 0330
Rancho Cordova, CA 95813-6000

STATEMENT OF COMPLIANCE

Study G98BA06.702 was conducted in compliance with the US FDA Good Laboratory Practice Regulations as published in 21 CFR 58, the US EPA GLP Standards 40 CFR 160 and 40 CFR 792, the UK GLP Compliance Regulations, the Japanese GLP Regulations and the OECD Principles of Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test or control article have not been determined by the testing facility.

Analyses to determine the uniformity, concentration, or stability of the test or control mixtures were not performed by the testing facility.

The stability of the test or control article under the test conditions has not been determined by the testing facility.

Richard H. C. San, Ph.D.
Study Director

1/27/99
Date

QUALITY ASSURANCE STATEMENT

Study Title: *IN VITRO* MAMMALIAN CELL GENE MUTATION TEST

Study Number: G98BA06.702

Study Director: Richard H. C. San, Ph.D.

This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 04 DEC 98, TO STUDY DIR 04 DEC 98, TO MGMT 04 DEC 98
PHASE: Protocol Review

INSPECT ON 15 DEC 98, TO STUDY DIR 15 DEC 98, TO MGMT 17 DEC 98
PHASE: Dilution of test and/or control material

INSPECT ON 20 JAN 99-21 JAN 99, TO STUDY DIR 21 JAN 99, TO MGMT 22 JAN 99
PHASE: Draft Report

INSPECT ON 27 JAN 99, TO STUDY DIR 27 JAN 99, TO MGMT 27 JAN 99
PHASE: Draft to Final Report

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Diane B. Madsen

1-27-99

Diane B. Madsen, B.S.
QUALITY ASSURANCE

DATE

In Vitro Mammalian Cell Gene Mutation Test
(L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

FINAL REPORT

Sponsor:	**Perchlorate Study Group** **Highway 50 and Aerojet Road** **Building 20019/Department 0330** **Rancho Cordova, CA 95813-6000**
Study Monitor:	**Michael F. Girard** **Perchlorate Study Group Representative**
Scientific Advisor:	**Michael L. Dourson, Ph.D., D.A.B.T.** **Toxicology Excellence for Risk Assessment**
Performing Laboratory:	**BioReliance** **9630 Medical Center Drive** **Rockville, MD 20850**
Test Article I.D.:	**ammonium perchlorate**
Test Article Lot No.:	**05006CQ**
Test Article Purity:	**99.999% (Provided by Sponsor)**
BioReliance Study No.:	**G98BA06.702**
Test Article Description:	**white, crystalline solid**
Storage Conditions:	**room temperature; protected from light and moisture**
Test Article Receipt:	**November 16, 1998**
Study Initiation:	**December 2, 1998**
Laboratory Manager:	**Jane J. Clarke, B.A.**
Study Director:	Richard H. C. San, Ph.D. 1/27/99 Date

TABLE OF CONTENTS

SUMMARY

The test article, ammonium perchlorate, was tested in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay in the absence and presence of Aroclor-induced rat liver S9. The preliminary toxicity assay was used to establish the dose range for the mutagenesis assay. The mutagenesis assay was used to evaluate the mutagenic potential of the test article.

Dimethyl sulfoxide (DMSO) was selected as the solvent of choice based on solubility of the test article and compatibility with the target cells. The test article was soluble in DMSO at 500 mg/mL, the maximum concentration tested.

In the preliminary toxicity assay, the maximum concentration of ammonium perchlorate in treatment medium was 5000 µg/mL. No visible precipitate was present at any concentration in treatment medium. Selection of dose levels for the mutation assay was based on reduction of suspension growth relative to the solvent control. Substantial toxicity, i.e., suspension growth of ≤50% of the solvent control, was not observed at any concentration with or without S9 activation.

Based on the results of the preliminary toxicity assay, the doses chosen for the mutagenesis assay ranged from 50 to 5000 µg/mL for both the non-activated and S9-activated cultures. No visible precipitate was present at any concentration in treatment medium. No cloned cultures exhibited mutant frequencies that were at least 55 mutants per 10^6 clonable cells over that of the solvent control. There was not a dose-response trend. Toxicity in the cloned cultures, i.e., total growth of ≤50% of the solvent control, was not observed at any doses without activation but was observed with S9 activation at doses of 4000 and 5000 µg/mL.

The trifluorothymidine-resistant colonies for the positive and solvent control cultures were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS positive control yielded the expected increase in small colonies, verifying the adequacy of the methods used to detect small colony mutants.

Under the conditions of this study, test article ammonium perchlorate was concluded to be negative in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay.

PURPOSE

The purpose of this study was to evaluate the mutagenic potential of the test article based on quantitation of forward mutations at the thymidine kinase locus of L5178Y mouse lymphoma cells.

CHARACTERIZATION OF TEST AND CONTROL ARTICLES

The test article, ammonium perchlorate, was received by BioReliance on November 16, 1998 and was assigned the code number 98BA06. The test article was characterized by the manufacturer as a white powder, which should be stored in a cool dry place. Its purity was given as 99.999%. Upon receipt, the test article was described as a white, crystalline solid and was stored at room temperature, protected from light and moisture.

The vehicle (solvent) used to deliver ammonium perchlorate to the test system was DMSO (CAS 67-68-5) obtained from Fisher.

Methyl methanesulfonate (MMS), CAS 66-27-3, lot # 09419LR, expiration date 5/01, was supplied by Aldrich Chemical Company and was used as the positive control for the non-activated test system at stock concentrations of 1000 and 2000 µg/mL. 7,12-Dimethyl-benz(a)anthracene (7,12-DMBA), CAS 57-97-6, lot # 85H0296, expiration date 1/99, was supplied by Sigma Chemical Company and was used at stock concentrations of 250 and 400 µg/mL as the positive control for the S9-activated test system.

MATERIALS AND METHODS

Test System

L5178Y cells, clone 3.7.2C, were obtained from Patricia Poorman-Allen, Glaxo Wellcome Inc., Research Triangle Park, NC. Each lot of cryopreserved cells was tested using the agar culture and Hoechst staining procedures and found to be free of mycoplasma contamination. Prior to use in the assay, L5178Y cells were cleansed of spontaneous $TK^{-/-}$ cells by culturing in a restrictive medium (Clive and Spector, 1975).

Metabolic Activation System

Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor-1254, 500 mg/kg, five days prior to sacrifice. The S9 was batch prepared and stored at ≤-70°C until used. Each bulk preparation of S9 was assayed for sterility and its ability to metabolize 2-aminoanthracene and 7,12-dimethyl-benz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100.

Immediately prior to use, the S9 was mixed with the cofactors and Fischer's Medium for Leukemic Cells of Mice with 0.1% Pluronics (F_0P) to contain 250 µL S9, 6.0 mg nicotinamide adenine dinucleotide phosphate (NADP), 11.25 mg DL-isocitric acid and 750 µL F_0P per mL of S9-activation mixture and kept on ice until used. The cofactor/F_0P mixture was filter sterilized and adjusted to pH 7.0 prior to the addition of S9. The formulation of the activation mixture is based on information from Turner *et al.* (1984). The final concentration of S9 in the treatment medium was 10%.

Solubility Test

A solubility test was conducted to select the solvent. The test was conducted using one or more of the following solvents in the order of preference as listed: distilled water, dimethyl sulfoxide, ethanol and acetone. The test article was tested to determine the solvent, selected in order of preference, that permitted preparation of the highest soluble or workable concentration, up to 500 mg/mL (the highest concentration tested).

Preliminary Toxicity Assay

The preliminary toxicity assay was used to establish the optimal dose levels for the mutagenesis assay. L5178Y cells were exposed to the solvent alone and nine concentrations of test article ranging from 0.5 to 5000 µg/mL in both the absence and presence of S9-activation.

Cell population density was determined 24 and 48 hours after the initial exposure to the test article. The cultures were adjusted to $3x10^5$ cells/mL after 24 hours only. Cultures with less than $3x10^5$ cells/mL were not adjusted. Toxicity was measured as suspension growth relative to the growth of the solvent controls.

Mutagenesis Assay

The mutagenesis assay was used to evaluate the mutagenic potential of the test article. L5178Y mouse lymphoma cells were exposed to the solvent alone and at least eight concentrations of test article in duplicate in both the absence and presence of S9. Positive controls, with and without S9-activation, were tested concurrently.

Treatment of the Target Cells

The mutagenesis assay was performed according to a protocol described by Clive and Spector (1975). Treatment was carried out in conical tubes by combining 6 x 106 L5178Y/TK+/- cells, 4 mL F0P medium or S9 activation mixture and 100 µL dosing solution of test or control article in solvent or solvent alone in a total volume of 10 mL. A total of at least eight concentrations of test article were tested in duplicate. The positive controls were treated with MMS (at final concentrations in treatment medium of 10 and 20 µg/mL) and 7,12-DMBA (at final concentrations in treatment medium of 2.5 and 4.0 µg/mL). Treatment tubes were gassed with 5±1% CO2 in air, capped tightly, and incubated with mechanical mixing for 4 hours at 37±1°C.

The preparation and addition of the test article dosing solutions were carried out under amber lighting and the cells were incubated in the dark during the exposure period. After the treatment period, the cells were washed twice with F0P or F0P supplemented with 10% horse serum and 2 mM L-glutamine (F10P). After the second wash, the cells were resuspended in F10P, gassed with 5±1% CO2 in air and placed on the roller drum apparatus at 37±1°C.

Expression of the Mutant Phenotype

For expression of the mutant phenotype, the cultures were counted using an electronic cell counter and adjusted to $3x10^5$ cells/mL at approximately 24 and 48 hours after treatment in 20 and 10 mL total volume, respectively. Cultures with less than $3x10^5$ cells/mL were not adjusted.

For expression of the $TK^{-/-}$ cells, cells were placed in cloning medium (C.M.) containing 0.23% granulated agar. Two flasks per culture to be cloned were labeled with the test article concentration, activation condition, and either TFT (trifluorothymidine, the selective agent) or V.C. (viable count). Each flask was prewarmed to 37±1°C, filled with 100 mL C.M., and placed in an incubator shaker at 37±1°C until used. The cells were centrifuged at 1000 rpm for 10 minutes and the supernatant was decanted. The cells were then diluted in C.M. to concentrations of $3x10^6$ cells/100 mL C.M. for the TFT flask and 600 cells/100 mL C.M. for the V.C. flask. After the dilution, 1.0 mL of stock solution of TFT was added to the TFT flask (final concentration of 3 µg/mL) and both this flask and the V.C. flask were placed on the shaker at 125 rpm and 37±1°C. After 15 minutes, the flasks were removed and 33 mL of the cell suspension was pipetted into each of three appropriately labeled petri dishes. To accelerate the gelling process, the plates were placed in cold storage (approximately 4°C) for approximately 30 minutes. The plates were then incubated at 37±1°C in a humidified 5±1% CO_2 atmosphere for 10-14 days.

Scoring Procedures

After the incubation period, the V.C. plates were counted for the total number of colonies per plate and the total relative growth determined. The TFT-resistant colonies were then counted for each culture with ≥10% total relative growth. The diameters of the TFT-resistant colonies for the positive and solvent controls and, in the case of a positive response, the test article-treated cultures were determined over a range of approximately 0.2 to 1.1 mm. The rationale for this procedure is as follows: Mutant L5178Y $TK^{-/-}$ colonies exhibit a characteristic frequency distribution of colony sizes. The precise distribution of large and small TFT-resistant mutant colonies appears to be the characteristic mutagenic "finger-print" of carcinogens in the L5178Y $TK^{+/-}$ system (Clive *et al.*, 1979; DeMarini *et al.*, 1989). Clive *et al.* (1979) and Hozier *et al.* (1981) have presented evidence to substantiate the hypothesis that the small colony variants carry chromosome aberrations associated with chromosome 11, the chromosome on which the TK locus is located in the mouse (Kozak and Ruddle, 1977). They suggested that large colony mutants received very localized damage, possibly in the form of a point mutation or small deletion within the TK locus, while small colony mutants received damage to collateral loci concordant with the loss of TK activity.

Evaluation of Results

The cytotoxic effects of each treatment condition were expressed relative to the solvent-treated control for suspension growth over two days post-treatment and for total growth (suspension growth corrected for plating efficiency at the time of selection). The mutant frequency (number of mutants per 10^6 surviving cells) was determined by dividing the average number of colonies in the three TFT plates by the average number of colonies in the three corresponding V.C. plates and multiplying by the dilution factor ($2x10^{-4}$) then multiplying by 10^6. For simplicity, this is described as: (Average # TFT colonies / average # VC colonies) x 200 in the tables.

In evaluation of the data, increases in mutant frequencies that occurred only at highly toxic concentrations (i.e., less than 10% total growth) were not considered biologically relevant. All conclusions were based on sound scientific judgement; however, the following criteria are presented as a guide to interpretation of the data (Clive *et al.*, 1995):

- The result was considered to induce a positive response if a concentration-related increase in mutant frequency was observed and one or more dose levels with 10% or greater total growth exhibited mutant frequencies of $\geq$100 mutants per 10^6 clonable cells over the background level.

- A result was considered equivocal if the mutant frequency in treated cultures was between 55 and 99 mutants per 10^6 clonable cells over the background level.

- Test articles producing fewer than 55 mutants per 10^6 clonable cells over the background level were concluded to be negative.

Criteria for a Valid Test

The following criteria must be met for the mutagenesis assay to be considered valid:

Negative Controls:

The spontaneous mutant frequency of the solvent control cultures must be within 20 to 100 TFT-resistant mutants per 10^6 surviving cells. The cloning efficiency of the solvent control group must be greater than 50%.

Positive Controls:

At least one concentration of each positive control must exhibit mutant frequencies of $\geq$100 mutants per 10^6 clonable cells over the background level. The colony size distribution for the MMS positive control must show an increase in both small and large colonies (Moore *et al.*, 1985; Aaron *et al.*, 1994).

Test Article-Treated Cultures:

A minimum of four analyzable concentrations with mutant frequency data will be required.

Archives

All raw data, protocol, and a copy of all reports will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance RAQA unit headquartered at:

BioReliance
14920 Broschart Rd.
Rockville, MD 20850

RESULTS AND DISCUSSION

Solubility Test

Dimethyl sulfoxide (DMSO) was selected as the solvent of choice based on solubility of the test article and compatibility with the target cells. The test article was soluble in DMSO at 500 mg/mL, the maximum concentration tested.

Preliminary Toxicity Assay

The results of the preliminary toxicity assay are presented in Table 1. The maximum dose tested in the preliminary toxicity assay was 5000 µg/mL. No visible precipitate was present at any dose level in treatment medium. The osmolality of the solvent control was 447 mmol/kg and the osmolality of the highest soluble dose, 5000 µg/mL, was 462 mmol/kg. Suspension growth relative to the solvent controls was 89% at 5000 µg/mL without activation and 72% at 5000 µg/mL with S9 activation. Based on the results of the toxicity test, the doses chosen for the mutagenesis assay ranged from 50 to 5000 µg/mL for both the non-activated and S9-activated cultures.

Mutagenesis Assay

The results of the mutagenesis assay are presented in Tables 2 through 5. Colony size distributions are presented in Figures 1 and 2. No visible precipitate was present at any dose level in treatment medium. In the non-activated system, cultures treated with concentrations of 1000, 2000, 3000, 4000 and 5000 µg/mL were cloned and produced a range in suspension growth of 61% to 98%. In the S9-activated system, cultures treated with concentrations of 1000, 2000, 3000, 4000 and 5000 µg/mL were cloned and produced a range in suspension growth of 14% to 80%.

No cloned cultures exhibited mutant frequencies that were at least 55 mutants per 10^6 clonable cells over that of the solvent control. A dose-response trend was not observed in the non-

activated or S9-activated systems. The total growths ranged from 69% to 92% for the non-activated cultures at concentrations of 1000 to 5000 µg/mL and 13% to 85% for the S9-activated cultures at concentrations of 1000 to 5000 µg/mL.

The TFT-resistant colonies for the positive and solvent control cultures were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS positive control yielded the expected increase in small colonies, verifying the adequacy of the methods used to detect small colony mutants.

CONCLUSION

All criteria for a valid study were met as described in the protocol. The results of the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay indicate that, under the conditions of this study, ammonium perchlorate was concluded to be negative.

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TABLE 1

PRELIMINARY TOXICITY ASSAY USING ammonium perchlorate

Test Article Concentration (µg/mL)	Cell Concentration (X10^6)[a] Day 1	Day 2	Suspension Growth Total[b]	% of Control[c]
WITHOUT ACTIVATION				
Solvent 1	0.921	1.343	13.7	
Solvent 2	0.915	1.297	13.2	
.5	0.901	1.362	13.6	101
1.5	0.923	1.357	13.9	103
5	0.863	1.373	13.2	98
15	0.827	1.392	12.8	95
50	0.872	1.323	12.8	95
150	0.926	1.282	13.2	98
500	0.862	1.359	13.0	97
1500	0.895	1.281	12.7	95
5000	0.732	1.469	11.9	89
WITH S-9 ACTIVATION				
Solvent 1	0.663	1.292	9.5	
Solvent 2	0.650	1.333	9.6	
.5	0.686	1.368	10.4	109
1.5	0.693	1.379	10.6	111
5	0.700	1.349	10.5	110
15	0.652	1.307	9.5	99
50	0.663	1.341	9.9	103
150	0.647	1.316	9.5	99
500	0.661	1.333	9.8	102
1500	0.606	1.413	9.5	99
5000	0.507	1.224	6.9	72

Solvent = DMSO

1 and 2 are duplicate cultures

[a] - Cultures containing <0.3x10^6 cells/mL on day 1 and 2 are considered as having 0% total suspension growth.

[b] - Total suspension growth = (Day 1 cell conc. / 0.3x10^6 cells/mL) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

[c] - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

TABLE 2

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH ammonium perchlorate IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)		TFT Colonies Counts			Mean	VC Colonies Counts				Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	22	21	24	22 ±1	191	178	170	180	±9	25		
Solvent	2	20	17	25	21 ±3	179	140	172	164	±17	25		
Mean Solvent Mutant Frequency= 25													
1000	A	27	17	15	20 ±5	149	176	148	158	±13	25	0	83
1000	B	13	21	24	19 ±5	157	156	131	148	±12	26	1	84
2000	A	28	26	14	23 ±6	168	155	159	161	±5	28	3	81
2000	B	18	22	19	20 ±2	172	181	169	174	±5	23	-2	92
3000	A	25	25	25	25 ±0	172	158	163	164	±6	30	5	90
3000	B	24	24	26	25 ±1	153	177	181	170	±12	29	4	85
4000	A	19	14	14	16 ±2	190	179	197	189	±7	17	-8	86
4000	B	31	20	24	25 ±5	205	184	207	199	±10	25	0	92
5000	A	17	24	25	22 ±4	195	183	157	178	±16	25	0	69
5000	B	24	34	32	30 ±4	203	188	189	193	±7	31	6	69
Positive Control - Methyl Methanesulfonate (µg/mL)													
10		106	139	159	135 ±22	114	110	114	113	±2	239	214	48
20		106	127	119	117 ±9	35	40	46	40	±4	582	557	12

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

TABLE 3

TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH ammonium perchlorate IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)		Cell Concentration (X 10^6)[a] Day 1	Day 2	Susp Growth Total[b]	%Cntl[c]	Cloning Growth Avg VC	%Cntl[d]	% Total Growth[e]
Solvent	1	1.438	1.362	21.8		180		
Solvent	2	1.446	1.270	20.4		164		
1000	A	1.229	1.397	19.1	90	158	92	83
1000	B	1.301	1.425	20.6	98	148	86	84
2000	A	1.236	1.333	18.3	87	161	94	81
2000	B	1.311	1.306	19.0	90	174	101	92
3000	A	1.200	1.492	19.9	94	164	96	90
3000	B	1.187	1.367	18.0	85	170	99	85
4000	A	1.131	1.318	16.6	79	189	110	86
4000	B	1.135	1.323	16.7	79	199	116	92
5000	A	1.062	1.193	14.1	67	178	104	69
5000	B	1.068	1.085	12.9	61	193	113	69
Positive Control - Methyl Methanesulfonate (µg/mL)								
10		1.232	1.134	15.5	74	113	66	48
20		1.081	0.884	10.6	50	40	23	12

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

[a] - Cultures containing <0.3x10^6 cells/mL on day 1 and 2 are considered as having 0% total suspension growth.

[b] - Total suspension growth = (Day 1 cell conc. / 0.3x10^6 cells/mL) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

[c] - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

[d] - % control cloning growth = (average V.C. of treated culture / average V.C. of solvent control) x 100

[e] - % total growth = (% suspension growth x % cloning growth) / 100

TABLE 4

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH ammonium perchlorate IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)		TFT Colonies Counts			Mean	VC Colonies Counts				Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	21	28	30	26 ±4	150	130	157	146	±11	36		
Solvent	2	24	34	50	36 ±11	163	178	163	168	±7	43		
Mean Solvent Mutant Frequency= 40													
1000	A	24	30	18	24 ±5	186	208	166	187	±17	26	-14	80
1000	B	15	19	28	21 ±5	158	157	181	165	±11	25	-15	85
2000	A	35	35	25	32 ±5	190	173	153	172	±15	37	-3	65
2000	B	21	24	26	24 ±2	199	170	207	192	±16	25	-15	82
3000	A	35	38	33	35 ±2	192	165	169	175	±12	40	1	58
3000	B	28	22	34	28 ±5	186	176	186	183	±5	31	-9	64
4000	A	38	32	33	34 ±3	169	172	150	164	±10	42	2	41
4000	B	33	28	34	32 ±3	183	185	175	181	±4	35	-5	42
5000	A	38	34	47	40 ±5	135	137	142	138	±3	57	18	13
5000	B	+	40	50	45 ±4	191	162	173	175	±12	51	12	21
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)													
2.5		135	129	136	133 ±3	120	129	137	129	±7	207	168	65
4		171	166	189	175 ±10	130	111	99	113	±13	309	270	42

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

\+ - Culture lost to contamination

[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

TABLE 5

TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH ammonium perchlorate IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)		Cell Concentration (X 10^6)[a] Day 1	Day 2	Susp Growth Total[b]	%Cntl[c]	Cloning Growth Avg VC	%Cntl[d]	% Total Growth[e]
Solvent	1	1.291	1.443	20.7		146		
Solvent	2	1.315	1.503	22.0		168		
1000	A	1.150	1.129	14.4	68	187	119	80
1000	B	1.225	1.256	17.1	80	165	105	85
2000	A	1.014	1.114	12.5	59	172	110	65
2000	B	1.060	1.218	14.3	67	192	122	82
3000	A	0.919	1.088	11.1	52	175	112	58
3000	B	0.845	1.248	11.7	55	183	116	64
4000	A	0.685	1.108	8.4	40	164	104	41
4000	B	0.642	1.083	7.7	36	181	115	42
5000	A	0.293	0.919	3.1	14	138	88	13
5000	B	0.412	0.866	4.0	19	175	112	21
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)								
2.5		1.087	1.397	16.9	79	129	82	65
4		0.949	1.188	12.5	59	113	72	42

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

[a] - Cultures containing <0.3x10^6 cells/mL on day 1 and 2 are considered as having 0% total suspension growth.

[b] - Total suspension growth = (Day 1 cell conc. / 0.3x10^6 cells/mL) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

[c] - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

[d] - % control cloning growth = (average V.C. of treated culture / average V.C. of solvent control) x 100

[e] - % total growth = (% suspension growth x % cloning growth) / 100

Figure 1

Colony Size Distribution in the Absence of Metabolic Activation

(Positive Control Compared with Solvent Control)

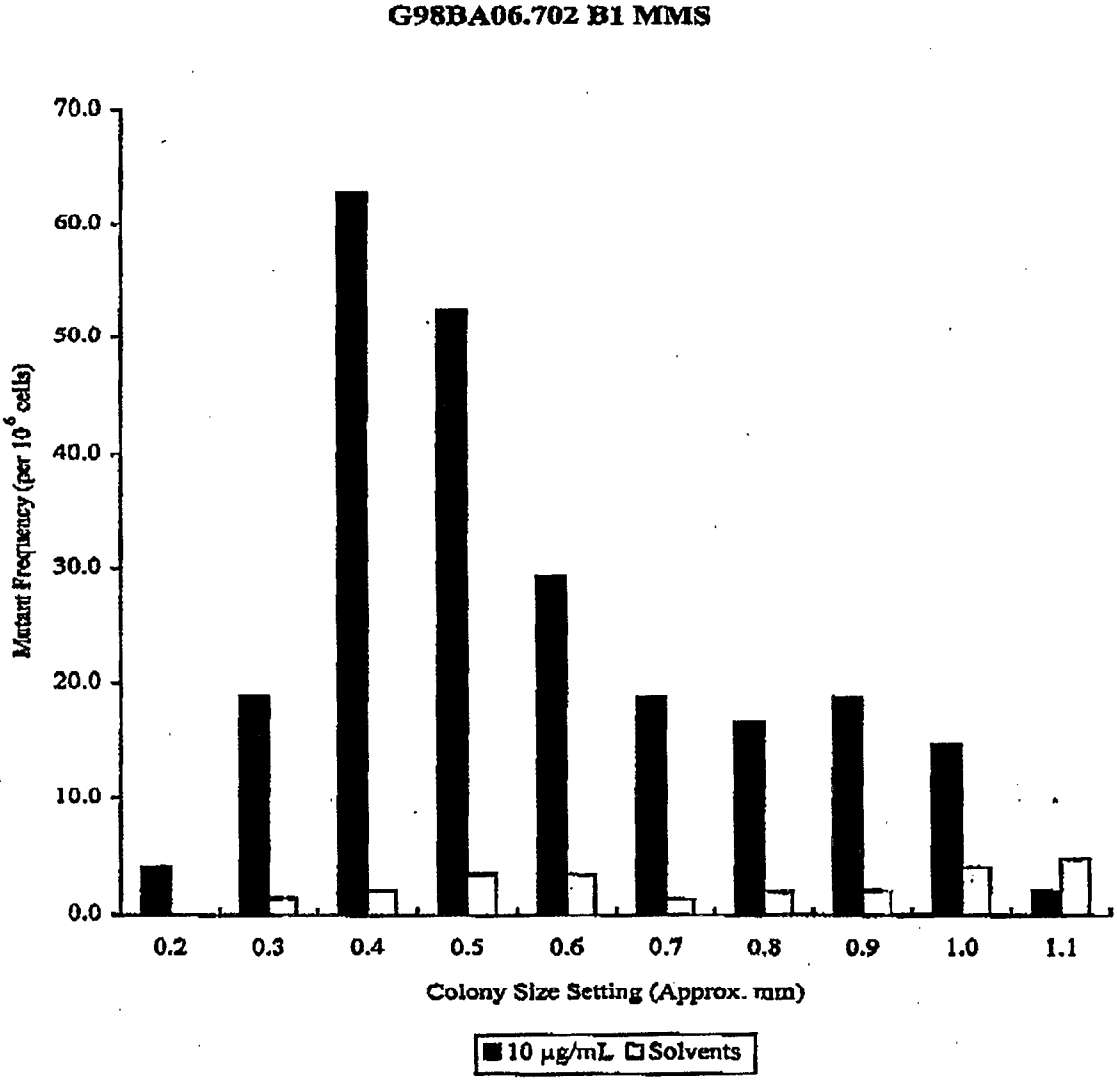

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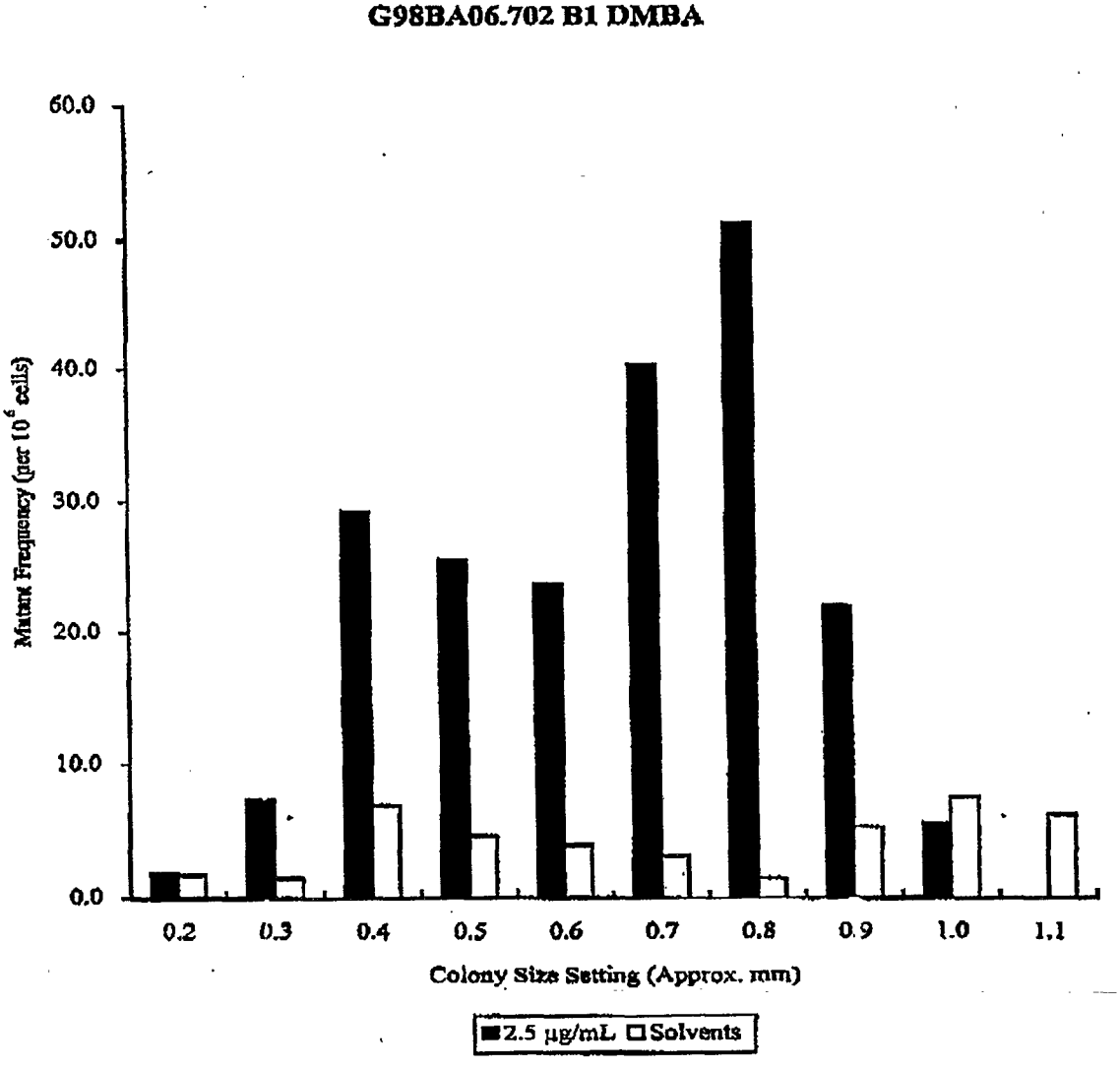
Figure 2

Colony Size Distribution in the Presence of Metabolic Activation

(Positive Control Compared with Solvent Control)



APPENDIX I

Historical Control Data

Mouse Lymphoma Historical Control Data

1995-1997

	Non-activated			S9-Activated		
	Solvent Control	20 µg/mL MMS	10 µg/mL MMS	Solvent Control	4.0µg/mL DMBA	2.5µg/mL DMBA
Mean MF	35.7	655.3	336.0	58.0	453.2	269.8
SD	10.3	293.3	128.5	18.6	158.5	95.1
Maximum	68.0	2400.0	729.0	100.0	1029.0	1048.0
Minimum	20.0	198.0	128.0	28.0	222.0	141.0

Solvent control (Fischer's medium, distilled water, saline, DMSO, ethanol, acetone or vehicle supplied by Sponsor)

MMS Methyl methanesulfonate
DMBA Dimethylbenz(a)anthracene
MF Mutant frequency per 10^6 clonable cells
SD Standard deviation

APPENDIX II

Study Protocol

BioReliance Study Number: G98BA06.702

In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

1.0 PURPOSE

The purpose of this study is to evaluate the mutagenic potential of the test article based on quantitation of forward mutations at the thymidine kinase locus of L5178Y mouse lymphoma cells.

2.0 SPONSOR

2.1	Name:	**Perchlorate Study Group**
2.2	Address:	Highway 50 and Aerojet Road Building 20019/Department 0330 Rancho Cordova, CA 95813-6000
2.3	Study Monitor:	Michael F. Girard Perchlorate Study Group Representative Telephone: (916) 355-2945 Telefax: (916) 355-6145
2.4	Scientific Advisor:	Michael L. Dourson, Ph.D., DABT Toxicology Excellence for Risk Assessment 4303 Hamilton Ave. Cincinnati, OH 45223 Telephone: (513) 542-7475 Telefax: (513) 542-7487
2.5	Sponsor Project #:	NP

3.0 IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

3.1	Test Article:		**Ammonium perchlorate**
3.2	Controls:	Negative:	Test article solvent (or vehicle)
		Positive:	Methyl methanesulfonate (MMS) 7,12-dimethylbenz(a)anthracene (DMBA)

3.3 Determination of Strength, Purity, etc.

Unless alternate arrangements are made, the testing facility at BioReliance will not perform analysis of the dosing solutions. The Sponsor will be directly responsible

for determination and documentation of the analytical purity and composition of the test article, and the stability and strength of the test article in the solvent (or vehicle).

3.4 Test Article Retention Sample

The retention of a reserve sample of the test article will be the responsibility of the Sponsor.

4.0 TESTING FACILITY AND KEY PERSONNEL

4.1	Name:	Toxicology Testing Facility BioReliance
4.2	Address:	9630 Medical Center Drive Rockville, MD 20850
4.3	Study Director:	Richard H. C. San, Ph.D.

5.0 TEST SCHEDULE

5.1 Proposed Experimental Initiation Date: 12/7/98

5.2 Proposed Experimental Completion Date: 1/19/99

5.3 Proposed Report Date: 1/28/99

6.0 TEST SYSTEM

L5178Y/TK$^{+/-}$ mouse lymphoma cells are heterozygous at the normally diploid thymidine kinase (TK) locus. L5178Y/TK$^{+/-}$, clone 3.7.2C, were received from Patricia Poorman-Allen, Glaxo Wellcome Inc., Research Triangle Park, North Carolina. Each freeze lot of cells has been tested and found to be free of mycoplasma contamination. This system has been demonstrated to be sensitive to the mutagenic activity of a variety of chemicals.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

The mammalian mutation assay will be performed by exposing duplicate cultures of L5178Y/TK$^{+/-}$ cells to a minimum of eight concentrations of test article as well as positive and negative (solvent) controls. Exposures will be for 4 hours in the presence and absence of an S9 activation system. Following a two day expression period, with daily cell population adjustments, cultures demonstrating 0% to 90% growth inhibition will be cloned, in triplicate, in restrictive medium containing soft agar to select for the mutant phenotype. After a 10 to 14 day selection period, mutant colonies will be enumerated. The mutagenic potential of the test article will be measured by its ability to induce TK$^{-/-} \rightarrow$ TK$^{+/-}$ mutations. For those test articles demonstrating a positive response, mutant colonies will be sized as an indication of mechanism of action.

7.1 Selection of Solvent

Unless the Sponsor has indicated the test article solvent, a solubility determination will be conducted to measure the maximum soluble concentration in a variety of solvents. Solvents compatible with this test system, in order of preference, include, but are not limited to, culture medium or distilled water (CAS 7732-18-5), dimethyl sulfoxide (CAS 67-68-5), ethanol (CAS 64-17-5) and acetone CAS 67-64-1). The solvent of choice will be that solvent, selected in order of preference, that permits preparation of the highest soluble stock concentration, up to a maximum of 500 mg/ml.

7.2 Dose Selection

In the preliminary toxicity test, L5178Y/TK$^{+/-}$ cells will be exposed to solvent alone and to at least nine concentrations of test article, the highest concentration being the lowest insoluble dose in treatment medium but not to exceed 5000 µg/ml. The pH of the treatment medium will be adjusted, if necessary, to maintain a neutral pH in the treatment medium. The osmolality of the highest soluble treatment condition will also be measured. After a 4-hour treatment in the presence and absence of S9 activation, cells will be washed twice with F_0P (Fischer's Media for Leukemic Cells of Mice with 0.1% Pluronics) or $F_{10}P$ (F_0P supplemented with 10% horse serum and 2mM L-glutamine) and cultured in suspension for two days post-treatment, with cell concentration adjustment on the first day.

Selection of dose levels for the mutation assay will be based on reduction of suspension growth after treatment in the preliminary toxicity test. Unless specified otherwise by the Sponsor, the high dose for the mutation assay will be that concentration exhibiting approximately 100% growth inhibition. The low dose will be selected to exhibit 0% growth inhibition. For freely soluble, non-toxic test articles, the highest concentration will be 5000 µg/ml. For relatively insoluble, non-toxic test articles, the highest concentration will be the lowest insoluble dose in treatment medium but not to exceed 5000 µg/ml. In all cases, precipitation will be evaluated at the beginning and at the end of the treatment period using the naked eye (ICH, 1996).

7.3 Route and Frequency of Administration

Cell cultures will be treated for 4 hours by way of a vehicle compatible with the system, both in the presence and absence of metabolic activation. This technique of administration has been demonstrated to be effective in the detection of chemical mutagens in this system.

7.4 Exogenous Metabolic Activation

Aroclor 1254-induced rat liver S9 will be used as the metabolic activation system. The source of S9 will be adult male Sprague-Dawley rats induced by a single injection of Aroclor 1254 at a dose level of 500 mg/kg body weight five days prior

to sacrifice. The S9 will be batch prepared and stored frozen at approximately -70°C until used.

Immediately prior to use, the S9 will be thawed and mixed with a cofactor pool to contain 11.25 mg DL-isocitric acid, 6 mg NADP, and 0.25 ml S9 homogenate per ml in F_0P. The S9 mix will be adjusted to pH 7.

7.5 Controls

7.5.1 Negative Control

The solvent (or vehicle) for the test article will be used as the negative control.

7.5.2 Positive Controls

Methyl methanesulfonate (MMS) will be used at two concentrations of 10 and 20 µg/ml as the positive control for the non-activated test system. For the S9-activated system, 7,12-dimethylbenz(a)anthracene (DMBA) will be used at two concentrations of 2.5 and 4.0 µg/ml.

7.6 Preparation of Target Cells

Prior to use in the assay, L5178Y/TK$^{+/-}$ cells will be cleansed to reduce the frequency of spontaneously occurring TK$^{-/-}$ cells. Using the procedure described by Clive and Spector (1975), L5178Y cells will be cultured for 24 hours in the presence of thymidine, hypoxanthine, methotrexate and glycine to poison the TK$^{-/-}$ cells.

L5178Y/TK$^{+/-}$ cells will be prepared at 1 x 10^6 cells/ml in 50% conditioned $F_{10}P$ and 50% F_0P. If cultures are to be treated with more than 100 µl of test article dosing solution, the cell concentration may be adjusted.

7.7 Identification of the Test System

Using a permanent marking pen, the treatment tubes will be identified by the study number and a code system to designate the treatment condition and test phase.

7.8 Treatment of Target Cells

Treatment will be carried out in conical tubes by combining 100 µl dosing solution of test or control article in solvent or solvent alone, 4 ml F_0P medium or S9 activation mixture with 6 x 10^6 L5178Y/TK$^{+/-}$ cells in a total volume of 10 ml. A minimum of eight concentrations of test article will be tested in duplicate. All pH adjustments will be performed prior to adding S9 or target cells to the treatment medium. Volumes of test article dosing solution in excess of 100 µl may be used if required to achieve the target final concentration in treatment medium. Treatment

tubes will be gassed with 5±1% CO_2 in air, capped tightly, and incubated with mechanical mixing for 4 hours at 37±1°C. The preparation and addition of the test article dosing solutions will be carried out under amber lighting and the cells will be incubated in the dark during the 4-hour exposure period.

7.9 Expression of the Mutant Phenotype

At the end of the exposure period, the cells will be washed twice with F_0P or $F_{10}P$ and collected by centrifugation. The cells will be resuspended in 20 ml $F_{10}P$, gassed with 5±1% CO_2 in air and cultured in suspension at 37±1°C for two days following treatment. Cell population adjustments to 0.3 x 10^6 cells/ml will be made at 24 and 48 hours.

7.10 Selection of the Mutant Phenotype

For selection of the trifluorothymidine (TFT)-resistant phenotype, cells from a minimum of five non-activated and five S9-activated test article concentrations demonstrating from 0% to 90% suspension growth inhibition will be plated into three replicate dishes at a density of 1 x 10^6 cells/100mm plate in cloning medium containing 0.23% agar and 2-4 μg TFT/ml. For estimation of cloning efficiency at the time of selection, 200 cells/100mm plate will be plated in triplicate in cloning medium free of TFT (viable cell (VC) plate). Plates will be incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 for 10-14 days.

The total number of colonies per plate will be determined for the VC plates and the total relative growth calculated. The total number of colonies per TFT plate will then be determined for those cultures with ≥10% total growth. Colonies are enumerated using an automatic counter; if the automatic counter cannot be used, the colonies will be counted manually. The diameters of the TFT colonies from the positive control and solvent control cultures will be determined over a range of approximately 0.2 to 1.1 mm. In the event the test article demonstrates a positive response, the diameters of the TFT colonies for at least one dose level of the test article (the highest positive concentration) will be determined over a range of approximately 0.2 to 1.1 mm.

7.11 Independent Repeat Assay

Verification of a clear positive response will not be required (OECD Guideline 476, ICH 1997). For equivocal and negative results, the Sponsor will be consulted regarding the need for an independent repeat assay.

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

8.1 Negative Controls

The spontaneous mutant frequency of the solvent (or vehicle) control cultures must be within 20 to 100 TFT-resistant mutants per 10^6 surviving cells. The cloning efficiency of the solvent (or vehicle) control group must be greater than 50%.

8.2 Positive Controls

At least one concentration of each positive control must exhibit mutant frequencies of $\geq$100 mutants per 10^6 clonable cells over the background level. The colony size distribution for the MMS positive control must show an increase in both small and large colonies (Moore *et al.*, 1985; Aaron *et al.*, 1994).

8.3 Test Article-Treated Cultures

A minimum of four analyzable concentrations with mutant frequency data will be required.

9.0 EVALUATION OF TEST RESULTS

The cytotoxic effects of each treatment condition are expressed relative to the solvent-treated control for suspension growth over two days post-treatment and for total growth (suspension growth corrected for plating efficiency at the time of selection). The mutant frequency for each treatment condition is calculated by dividing the mean number of colonies on the TFT-plates by the mean number of colonies on the VC-plates and multiplying by the dilution factor (2×10^{-4}), and is expressed as TFT-resistant mutants per 10^6 surviving cells.

In evaluation of the data, increases in mutant frequencies which occur only at highly toxic concentrations (i.e., less than 10% total growth) are not considered biologically relevant. All conclusions will be based on sound scientific judgement; however, the following criteria are presented as a guide to interpretation of the data (Clive *et al.*, 1995):

- The result will be considered to induce a positive response if a concentration-related increase in mutant frequency is observed and one or more dose levels with 10% or greater total growth exhibit mutant frequencies of $\geq$100 mutants per 10^6 clonable cells over the background level.

- A result will be considered equivocal if the mutant frequency in treated cultures is between 55 and 99 mutants per 10^6 clonable cells over the background level.

- Test articles producing fewer than 55 mutants per 10^6 clonable cells over the background level will be concluded to be negative.

10.0 REPORT

A report of the results of this study will be prepared by the Testing Laboratory and will accurately describe all methods used in the generation and analysis of data.

Results presented will include, but not be limited to:

- test substance: identification and CAS no., if known; physical nature and purity, if known; physicochemical properties relevant to the conduct of the study, if known; stability of test article, if known.
- solvent/vehicle: justification for choice of vehicle; solubility and stability of test article in solvent/vehicle, if known.
- cell type used, number of cultures, methods for maintenance of cell cultures
- rationale for selection of concentrations and number of cultures
- test conditions: composition of media, CO_2 concentration, concentration of test substance, vehicle, incubation temperature, incubation time, duration of treatment, cell density during treatment, type of metabolic activation system, positive and negative controls, length of expression period, selective agent
- method used to enumerate numbers of viable and mutant colonies and the number of colonies in each plate
- dose-response relationship, if applicable
- distribution of the mutant colony diameter for the solvent and positive controls and, when the test article induces a positive response, for at least one dose level of the test article (the highest positive concentration)
- positive and solvent control historical data

11.0 RECORDS AND ARCHIVES

Upon completion of the final report, all raw data and reports will be maintained in the archives of BioReliance, Rockville, MD in accordance with the relevant Good Laboratory Practice Regulations.

12.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

This protocol has been written to comply with OECD Guideline for the Testing of Chemicals, Guideline 476 (*In Vitro* Mammalian Cell Gene Mutation Test), July 1997, and with the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals, S2A document recommended for

adoption at step 4 of the ICH process on July 19, 1995. Federal Register 61:18198-18202. April 24, 1996.

This study will be performed in compliance with the provisions of the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies.

Will this study be submitted to a regulatory agency? yes

If so, to which agency or agencies? U.S. EPA, U.S DOD

Unless arrangements are made to the contrary, unused dosing solutions will be disposed of following administration to the test system and all residual test article will be disposed of following finalization of the report.

13.0 REFERENCES

Aaron, C.S., Bolcsfoldi, G., Glatt, H.-R., Moore, M., Nishi, Y., Stankowski, L., Theiss, J. and Thompson, E. (1994) Mammalian cell gene mutation assays working group report. Mutation Research 312:235-239.

Clive, D., Bolcsfoldi, G., Clements, J., Cole, J., Homna, M., Majeska, J., Moore, M., Muller, L., Myhr, B., Oberly, T., Oudelhkim, M., Rudd, C., Shimada, H., Sofuni, T., Thybaud, V. and Wilcox, P. (1995) Consensus agreement regarding protocol issues discussed during the mouse lymphoma workshop: Portland, Oregon, May 7, 1994. Environ. Molec. Mutagen. 25:165-168.

Clive, D. and Spector, J.F.S. (1975) Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. Mutation Research 31:17-29.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals. S2A document recommended for adoption at step 4 of the ICH process on July 19, 1995. Federal Register 61:18198-18202. April 24, 1996.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. S2B document recommended for adoption at step 4 of the ICH process on July 16, 1997. Federal Register 62:16026-16030, November 21, 1997.

Moore, M.M., Clive, D., Howard, B.E., Batson, A.G. and Turner, N.T. In situ analysis of trifluorothymidine-resistant (TFT^r) mutants of L5178Y/TK$^{+/-}$ mouse lymphoma cells. (1985) Mutation Research 151:147-159.

OECD Guideline for the Testing of Chemicals, Guideline 476 (*In Vitro* Mammalian Cell Gene Mutation Test), July 1997.

14.0 APPROVAL

Michael F. Girard — 11/19/98
Sponsor Study Monitor — Date

Michael L. Dourson, Ph.D., DABT — 11-10-98
Sponsor Scientific Advisor — Date

Richard H.C. San, Ph.D. — 12/2/98
BioReliance Study Director — Date

If submission to Japanese Regulatory Agency is indicated in section 12.0, BioReliance management will sign.

David Jacobson-Kram, Ph.D., DABT — Date
BioReliance Study Management

5.3 GENOTOXICITY ASSAYS

ManTech Environmental Technology, Inc., performed a battery of three genotoxicity assays (*Salmonella typhimurium*/microsome mutagenesis assay [Ames assay], the mouse lymphoma cell mutagenesis assay [L5178Y-TK test], and the in vivo mouse bone marrow micronucleus induction assay) with ammonium perchlorate to help determine its potential for various interactions with DNA and to gain insight on its possible carcinogenicity (ManTech Environmental Technology, Inc., 1998). To confirm the findings of ManTech Environmental Technology, the EPA requested the National Toxicology Program to also evaluate ammonium perchlorate in the Ames assay and the mouse bone marrow micronucleus test (NTP, 1999a). The sponsor (PSG) also had the mouse lymphoma assay repeated (BioReliance, 1999).

Ammonium Perchlorate was evaluated in the Ames assay (*Salmonella typhimurium*/ microsome mutagenesis assay), which is a well-defined assay for detection of carcinogens/ mutagens. It measures the reversion from a his^- (histidine independent) state induced by chemicals that cause base-pair changes or frameshift mutations in the genome of the organism (i.e., it measures for point mutations [e.g., substitution, addition, or deletion of one or a few DNA base pairs within a gene]). In this assay, bacteria are exposed to the test chemical with and without a metabolic activation system (Arochlor 1254-induced rat liver S9 with co-factors). The mutagenicity is evaluated by the increase in the number of revertant colonies. The L5178Y mouse-lymphoma assay is another short term in vitro assay to detect both point mutations and structural chromosomal changes. The in vivo mammalian micronucleus test detects the damage of chromosomes or of the mitotic apparatus caused by a clastogenic chemical in bone marrow cells (polychromatic erythrocyte [PCE] stem cells) of treated animals. Micronuclei are believed to be formed from chromosomes or chromosome fragments left behind during anaphase of mitosis. The induction of micronuclei indicates changes in either chromosome structure or number in bone marrow cells. ManTech Environmental Technology, Inc., performed this assay in Swiss-CD-1 mice and the NTP used B6C3F1 mice (NTP, 1999a). The micronucleus assay also was performed as part of the 90-day bioassay in Spraque-Dawley rats (Springborn Laboratories, Inc., 1998). This is an adequate series of tests to determine the mutagenic and clastogenic

(chromosomal breaking) potential of an agent. It should be noted that perchlorate is not likely to be mutagenic, given its physical and chemical properties (i.e., it is simply an anion). Although perchlorate is an oxidizing agent, it is not expected to produce oxidative DNA damage because of the kinetic considerations discussed in Chapter 2.

5.3.1 In Vitro Assays

Ammonium perchlorate was not found to be mutagenic in the *Salmonella typhimurium* (Ames assay) with and without Arochlor 1254-induced rat liver S9 activation by two separate laboratories (ManTech Environmental Technology, Inc., 1998; NTP, 1999b). In the ManTech study, ammonium perchlorate was dissolved in distilled water and tested at five concentrations (5,000, 2,500, 1,250, 625, and 312.5 µg/plate) in tester strains TA98, TA100, TA1535, and TA1537, without and with Arochlor 1254-induced rat liver S9 using the plate incorporation assay. Although this study was regarded as adequate, the EPA requested that Ames assay be repeated by the National Toxicology Program (NTP) to confirm the negative findings and to include additional tester strains (i.e., TA102 and TA104) which are able to detect a variety of oxidative mutagens. Therefore, NTP evaluated ammonium perchlorate in the Salmonella/Ames assay in tester strains TA98, TA100, TA1535, TA97, TA102, and TA104 (NTP, 1999b). Ammonium perchlorate was dissolved in distilled water and tested using the preincubation procedure at doses of 10,000, 3,333, 1,000, 333, and 100 µg/plate, with and without metabolic activation from Arochlor-induced rat and hamster livers. Ammonium perchlorate was neither toxic nor mutagenic under the conditions of the NTP assay.

The L5178Y/$tk^{+/-}$ mouse lymphoma assay also was used to evaluate the mutagenic and chromosomal breaking potential of ammonium perchlorate in vitro. Ammonium perchlorate was reported to be negative both in the absence and presence of rat Arochlor-induced S9 liver activation (ManTech Environmental Technology, Inc., 1998). Ammonium perchlorate was evaluated at 5.0, 2.5, 0.5, 0.25, 0.05, and 0.025 mg/mL without S9 activation, and at 2.5, 0.5, 0.25, 0.05, and 0.025 mg/mL with S9 activation. Although a small increase in mutation frequency was found in the absence of S9 activation at 2.5 mg/mL, which appeared to be statistically significant ($p < 0.05$) by the two-tail, Student's t-test, a repeat assay found no increase in

mutation frequency at this concentration compared with controls. Therefore, ammonium perchlorate is considered to be negative in the absence of S9 activation. Confidence in the negative findings without S9 activation is reinforced by the wide range of ammonium perchlorate concentrations evaluated. Although ammonium perchlorate also was reported as negative in the presence of S9 activation, the response of the positive control, 3-methyl cholanthrene (MCA), in the actual experiment was too low (182.6×10^{-6}) to be acceptable. The highest dose of ammonium perchlorate produced a mutation frequency of 194×10^{-6}. The MCA at 2.5 µg/mL should induce a mutation frequency of 300 to 350×10^{-6} or higher. Such a low positive control response weakens the confidence for the negative finding with S9 activation. In addition, the cloning efficiencies for the S9 test appear to be too high (143%), further reducing the confidence in a negative finding. Therefore, only the assays on ammonium perchlorate without S9 are considered unequivocally to be negative. Although perchlorate is not expected to be metabolized to a mutagenic intermediate, these S9 data are not of sufficient quality to support a clear negative-response conclusion.

Because of the problems described above, the sponsor (PSG) had the mouse lymphoma assay repeated. In this recent mouse lymphoma assay, ammonium perchlorate was evaluated at concentrations of 1000, 2000, 3000, 4000, and 5000 ug/ml without and with Arochlor 1254-induced rat liver S9 activation (BioReliance, 1999). No increase in mutant frequencies were found after treatment with perchlorate. The data are judged to be of sufficient quality to determine perchlorate to be nonmutagenic both with and without S9 activation. Although the background mutant frequency was low, particularly in the without S9 experiment, the data set still is considered to be overall very good., as well as internally consistent. The problems that were observed in the data generated by the first laboratory (ManTech Environmental Technology, Inc., 1998) are not present in the data from the BioReliance study.

5.3.2 In Vivo Assays

The potential for ammonium perchlorate to induce micronuclei was evaluated in mice and rats. Ammonium perchlorate was administered by drinking water gavage for 3 consecutive days to Swiss CD-1 mice (5 females and 5 males per dose group) at 1,000, 500, 250, 125, and

62.5 mg/kg-day (ManTech Environmental Technology, Inc., 1998). Twenty-four hours after the last dose, the mice were sacrificed, and the frequency of micronucleated cells were evaluated by counting 1,000 PCEs per animal. The assay was conducted in accordance with existing EPA FIFRA/TSCA testing guidelines. No increase in the frequency of micronuclei were found for any dose group. There is some uncertainty whether a maximum tolerated dose (MTD) was reached in this study. The study authors reported that at 2,000 mg/kg, 4 out of 6 animals died after one dosing of ammonium perchlorate. Typically, the assay is performed at 85% of the MTD, and the 1,000 mg/kg-day represents approximately 50% of the LD_{50}. There was no indication of toxicity to the bone marrow cells because the PCE/NCE ratio was not different from negative controls. Furthermore, the study authors did not report any indication of clinical signs of toxicity in the highest dose group. Despite a rebuttal submitted by Dourson (1998) on behalf of the sponsor (PSG), EPA remained concerned because of the importance of this test in the overall determination of the approach to be taken for the carcinogenicity assessment (i.e., to rule out direct genotoxicity).

The NTP agreed to expedite and repeat this test in response to an EPA request. The assay was performed by ip injection to ensure the greatest delivery to the bone marrow. Male B6C3F1 mice were treated with 125, 250, 500, 1,000, 1,500, and 2,000 mg/kg ammonium perchlorate in buffered saline, plus solvent and positive (cyclophosphamide) controls. Note that this study uses two dose groups higher than those used in the previous study (i.e., 1,500 and 2,000 mg/kg). Furthermore, use of ip injection as the route of administration would result in a direct delivery of the compound to the bone marrow cells versus drinking water gavage. Five mice per group were injected daily for 3 consecutive days and were sacrificed 24 h after the last injection; 2,000 PCEs were scored per animal for micronuclei. All animals in the 1,500- and 2,000-mg/kg groups died after the first ip injection, and 4/5 animals died in the 1,000-mg/kg group after the second ip injection. No increases in percent PCE were observed in any of the remaining test groups (125, 250, and 500 mg/kg). No bone marrow toxicity was seen as indicated by the percent of PCE. These results are interpreted to be consistent with those of the ManTech Environmental Technology, Inc. (1998) study that used gavage drinking water administration, and confirm that perchlorate does not induce micronuclei in rodents.

The 90-day subchronic bioassay using Spraque-Dawley rats also evaluated micronuclei induction (Springborn Laboratories, Inc., 1998). The frequency of micronuclei induction was examined in both the males and females after the 90-day sacrifice in the 10-mg/kg-day dose group of ammonium perchlorate administered by drinking water. Although there was no induction of micronuclei at this dose, 10 mg/kg-day does not appear to reach a MTD because there were no overt signs of toxicity, although the definition of MTD may be somewhat moot, given the changes in thyroid hormone economy and histopathology seen in the thyroids at that dose. There was significant reduction in the PCE/NCE ratio (i.e, an indicator of toxicity to the bone marrow cells).

5.3.3 Summary of Genotoxicity Battery Results

Negative results were reported in all genotoxicity assays conducted on ammonium perchlorate when evaluated by two independent laboratories. Ammonium perchlorate was not mutagenic in the Ames assay (with or without S9 activation). Negative results were also found in the mouse lymphoma gene mutation assay without and with S9 activation. Ammonium perchlorate did not induce chromosomal anomalies when evaluated for micronuclei induction in the bone marrow of mice when administered via drinking water gavage or i.p. injection No increases in micronuclei were found in Spraque-Dawley rats when evaluated from the 90-day study at the highest dose, which produced both thyroid hormone perturbations and follicular cell hyperplasia. It is concluded that ammonium perchlorate does not have the potential to be mutagenic or clastogenic. The in vitro and in vivo studies discussed above provide support for that conclusion. Therefore, mutagenicity is not considered a possible mode of carcinogenic action for this chemical.

February 1, 1999 EPA Assessment Submission

Attachment #2
Analysis of Brain Histopathology at 3 mg/kg-day
Argus (1998a) Neurodevelopmental Study

A. Argus 1/20/98 Data Submission (York, 1998f)
B. EPA analysis (Geller, 1999a)

ATTENTION PANEL MEMBER(S):

TOM ZOELLER

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
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November 20, 1998

Annie Jarabek
USEPA, National Center for
Environmental Assessment
3210 Highway 54, Catawba Bldg.
Research Triangle Park, NC 27709

Telephone: (919) 541-4847
Telefax: (919) 541-1818

RE: Protocol 1416-001 - Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats

Dear Ms. Jarabek:

Enclosed is a diskette containing the thyroid hormone data for the Fo generation adults and F1 generation pups supplied by AniLytics, as well as a summary table created by Argus to show the mean group values and identify which groups are significantly different than control values. Please note that there is an error in the data supplied by AniLytics. For the F1 generation females, pup number 3668 has been incorrectly identified as being in the 30.0 mg/kg/day dosage group, and should be 3.0 mg/kg/day. The summary table does reflect this correction. AniLytics has been made aware of this incorrect value and will make the necessary changes to their data.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Raymond G. York, Ph.D., DABT
Associate Director of Research
and Study Director

RGY:hmg
Enc.
Copies to: D. Mattie
M. Dourson

Protocol 1416-001: Summary of Thyroid Hormone Data

Fo Generation Rats:

Dosage Group	Dosage Level (mg/kg/day)	TSH (ng/mL)		T3 (ng/dL)		T4 (µg/dL)	
		Male Rats	Female Rats	Male Rats	Female Rats	Male Rats	Female Rats
I	0	1.530	2.054	72.547	57.770	4.641	2.126
II	0.3	1.353	2.213	87.389**	64.789	4.726	2.903**
III	3.0	1.487	1.990	88.452**	56.350	4.744	2.924**
IV	30	3.871**	2.174	78.570	60.373	3.578**	2.421

F1 Generation Pups:

Dosage Group	Dosage Level (mg/kg/day)	TSH (ng/mL)		T3 (ng/dL)		T4 (µg/dL)	
		Male Pups	Female Pups	Male Pups	Female Pups	Male Pups	Female Pups
I	0	1.237	1.120	105.897	105.954	4.403	4.270
II	0.3	.941**	1.188	111.150	109.922	4.615	4.865*
III	3.0	.877**	1.141	109.810	109.293	4.533	4.324
IV	30	1.270	1.301	107.398	97.581*	4.525	3.913

* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

Neurotoxicology Division, MD-74B

MEMORANDUM

Date: 27 January 1999

Subject: Analysis of the Brain Morphometry Data from the Neurobehavioral Developmental Study of Ammonium Perchlorate (Argus, 1998a)

From: Andrew M. Geller
Neurotoxicology Division, MD-74B
National Health Effects and Environmental Research Laboratory

To: Annie Jarabek
National Center for Environmental Assessment

Attached is the statistical analysis of the hormone data from the Argus Neurobehavioral Developmental Study (Argus Protocol #1613-002). Data was received from Argus on November 5, 1998 (York, 1998d) and imported in ASCII form to SAS for further analysis. I have attached a description of how the analyses were done, a description of results, and summary graphs.

Analyses of Brain Morphometry Data from Neurobehavioral Developmental Study (Argus, 1998a)

Summary: A memo from Argus Laboratories (York, 1998d) contains brain morphometry data from the control, 3 mg/kg/day and 10 mg/kg/day dose groups from the Neurobehavioral Developmental Study of ammonium perchlorate in the rat at post-natal day 12 in the F1 generation (Argus, 1998a). This memo adds the morphometric data from the 3 mg/kg/day data to that of the control and high dose (10 mg/kg/day) groups previously reported in Tables 1 and 2 of Appendix P (Argus, 1998a). This data had been requested by the USEPA after initial findings of a morphometric increase in the size of the corpus callosum in the high dose group relative to controls. At the time that the report on Perchlorate Environmental Contamination had been prepared for External Review, only the data from the corpus callosum had been re-analyzed by the USEPA (Crofton, 1998c). The results of analysis of the morphometry data from the other brain regions is reported here.

Data was analyzed using a 2-way analysis of variance, with dose and sex as independent variables. It is desirable in the analysis of developmental data to have litter information; since none was included in Appendix P (Argus, 1998a) or the memo (York, 1998d), it is possible that the effects of sex and litter are confounded.

Significant effects of dose were found in corpus callosum, hippocampal gyrus, anterior/posterior cerebellum, and caudate putamen. An effect of sex was also found in caudate putamen.

The corpus callosum showed an increase in size at the highest dose tested (10 mg/kg /day). The other significant dose effects were driven by effects at the 3.0 mg/kg/day dose group. There was a significant decrease in size in this dose group in hippocampal gyrus and caudate putamen and a significant increase in size in anterior/posterior cerebellum.

Data: All data were supplied in the form of a memo (York, 1998d). Data were keyed in and entered as ASCII files for analyses by SAS.

Data for dependent measures (brain weight, anterior/posterior cerebrum, anterior/posterior cerebellum, frontal cortex, parietal cortex, caudate putamen, corpus callosum, hippocampal gyrus, cerebellum, external germinal layer) were subjected to separate two-way ANOVAs. Treatment (dose) and sex were the independent between-subjects variables. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. Where there was a dose x sex interaction, separate one-way ANOVAs were run for each gender.

To correct for multiple comparisons the acceptable alpha for significance (for all interaction main effects tests) was corrected to 0.016 (alpha of 0.05 divided by the square root of the number of ANOVAs).

Data Analysis - Results:

Significant effects of dose were found in corpus callosum, hippocampal gyrus, anterior/posterior cerebellum, and caudate putamen (Figure 1). An effect of sex was also found in caudate putamen.

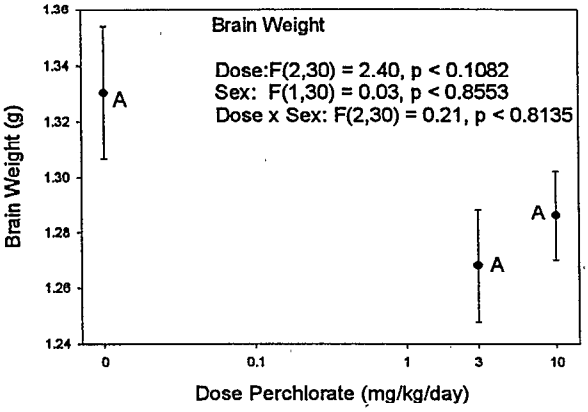
Corpus callosum showed an increase in size in the 10 mg/kg/day dose group, as previously reported in Crofton (1998c).

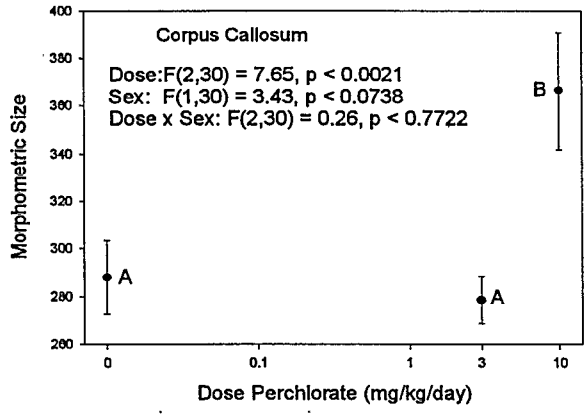
Hippocampal gyrus (12% less than control) and caudate putamen (7.3% less than control) showed a decrease in size at the 3 mg/kg/day dose, with no significant difference between control and high dose, yielding a U-shaped dose response. A/P cerebellum showed a significant increase in size in the 3 mg/kg/day group (13% greater than control), yielding an inverted U-shaped dose response function.

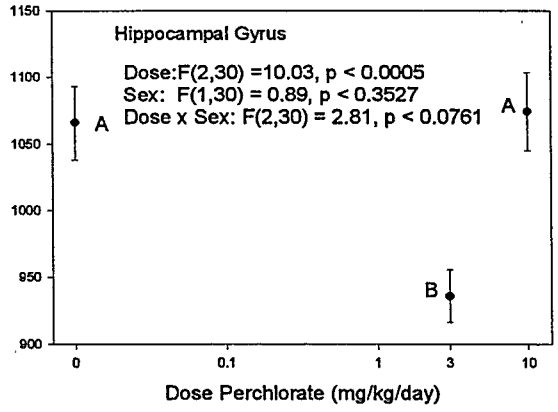
Inhibition of iodide uptake is highly non-linear and saturable, and therefore does not rule out the possibility of a U-shaped dose response. Until the PBPK modeling better characterizes this phenomenon, we are not requesting histopathological evaluation of brain sections at the next lower dose. This is pending commentary with respect to the potential for U-shaped dose response for changes in brain morphology with perchlorate exposure and other recommendations made at the external peer review. We do request, however, that the tissue samples be saved until a final decision is made on this matter.

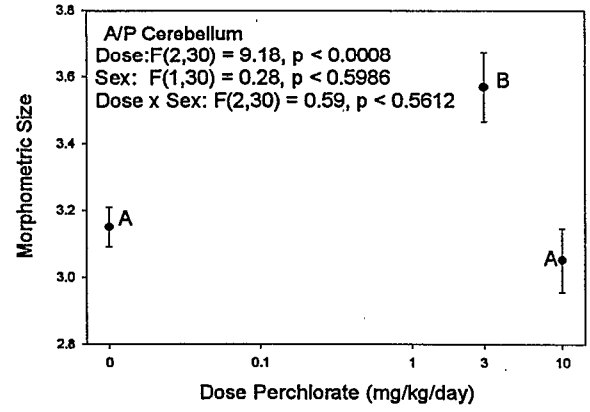
Neurobehavioral Developmental Study of Ammonium Perchlorate in Drinking Water (Argus, 1998a)

F1 Generation, PND12, Male and Female Combined.
Brain weight and morphometric size measurements for different brain regions. There is no effect on brain weight. Other plots show regions where significant effect of dose was found in 2-way analysis of variance (independent variables = dose, sex). Within each plot, means with different letters are significantly different ($p < 0.05$).









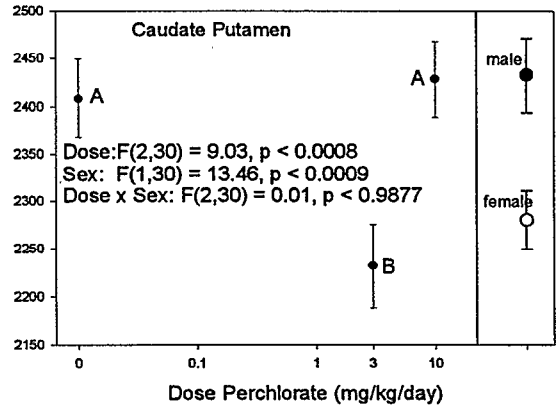


Figure 1

```
NOTE: Copyright (c) 1989-1996 by SAS Institute Inc., Cary, NC, USA.
NOTE: SAS (r) Proprietary Software Release 6.12  TS020
      Licensed to US ENVIRONMENTAL PROTECTION AGENCY, Site 0019614059.

NOTE: Running on ALPHASERVER Model 2100 5/300 Serial Number 80000000.


WARNING: Your system is scheduled to expire on February 18, 1999, which is 23 days from now. Please contact your installation
         representative to have your system renewed. The SAS system will no longer function on or after that date.
 Welcome to the NHEERL-RTP SAS Information Delivery System.


1          *THIS FILE IS FOUND AT [CRofton.THYROID.perchlorate]perchlorate_dn_pnd5.SAS;
2          *IT ANALYZES THE THYROID HORMONE DATA FROM THE WPAFB 90 DAY PERCHLORATE STUDY;
3
4
5          *INPUT DATA INTO SAS DATASET;
6          DATA RAW; INFILE '[GELLER.BMD]1613-002.Txt';
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
7               INPUT SEX$ DOSE$ RATNO BRAINWT CEREBRUM APCBLM FCORTEX PCORTEX
8                       CAUDPUT CORPCOL HIPPO CEREBLL XGEM;
9
10         * BRAINWT = TOTAL BRAIN WEIGHT;
11         * CEREBRUM = ANTER/POST CEREBRUM;
12         * APCBLM = ANT/POST CEREBELLUM;
13         * FCORTEX = FRONTAL CORTEX;
14         * PCORTEX = PARIETAL CORTEX;
15         * CAUDPUT = CAUDATE PUTAMEN;
16         * CORPCOL = CORPUS CALLOSUM;
17         * HIPPO = HIPPOCAMPAL GYRUS;
18         * CEREBLL = CEREBELLUM;
19         * XGEM = EXT GERM LAYER;
20
21         *ASSIGN TREATMENT VALUES TO DOSE CODES;
22              IF DOSE = '1' THEN TRT = '1--------CONTROL';
23              IF DOSE = '2' THEN TRT = '2--0.1_mg/kg/day';
24              IF DOSE = '3' THEN TRT = '3--1.0_mg/kg/day';
25              IF DOSE = '4' THEN TRT = '4--3.0_mg/kg/day';
26              IF DOSE = '5' THEN TRT = '5-10.0_mg/kg/day';
27

NOTE: The infile '[GELLER.BMD]1613-002.Txt' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]1613-002.TXT

NOTE: 36 records were read from the infile '[GELLER.BMD]1613-002.Txt'.
      The minimum record length was 73.
      The maximum record length was 73.
NOTE: The data set WORK.RAW has 36 observations and 14 variables.

28                 PROC PRINT;
```

```
WARNING: The BASE Product product with which PRINT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
29                    TITLE "PERCHLORATE NEURODEVELOPMENTAL ARGUS 1613-002 - RAW DATA";
30
31          *SORT DATA BY TRT -- THEN GET MEANS;
32
33
12                                                  The SAS System                              15:56 Tuesday, January 26, 1999


NOTE: The PROCEDURE PRINT printed page 1.

33            PROC SORT; BY TRT;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
34

NOTE: The data set WORK.RAW has 36 observations and 14 variables.

34            PROC MEANS N MEAN STDERR MIN MAX STD VAR CV; BY TRT;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
35                    VAR BRAINWT CEREBRUM APCBLM FCORTEX PCORTEX CAUDPUT
36                           CORPCOL HIPPO CEREBLL XGEM;;
37                    TITLE1 "PERCHLORATE NEURODEVELOPMENTAL ARGUS 1613-002 - RAW DATA";
38                    TITLE2 "GROUP MEANS BY TREATMENT";
39
40          *SORT DATA BY TRT AND GENDER -- THEN GET MEANS;
41
42

NOTE: The PROCEDURE MEANS printed page 2.

42            PROC SORT; BY TRT SEX;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
43

NOTE: The data set WORK.RAW has 36 observations and 14 variables.

43            PROC MEANS N MEAN STDERR MIN MAX STD VAR CV; BY TRT SEX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
44                    VAR BRAINWT CEREBRUM APCBLM FCORTEX PCORTEX CAUDPUT
45                           CORPCOL HIPPO CEREBLL XGEM;
46                    TITLE1 "PERCHLORATE NEURODEVELOPMENTAL ARGUS 1613-002 - RAW DATA";
47                    TITLE2 "GROUP MEANS BY GENDER AND TREATMENT";
48
49          *RUN ONE WAY ANOVAs FOR ALL VARIABLES;
50

NOTE: The PROCEDURE MEANS printed pages 3-4.
```

```
50          PROC SORT; BY TRT SEX;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
51

NOTE: Input data set is already sorted, no sorting done.

51          PROC GLM;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
52                  CLASSES TRT SEX;
53                  MODEL BRAINWT CEREBRUM APCBLM FCORTEX PCORTEX CAUDPUT
54                         CORPCOL HIPPO CEREBLL XGEM = TRT|SEX;
55                  MEANS TRT/TUKEY LINES;
13                                                  The SAS System                              15:56 Tuesday, January 26, 1999

56                  TITLE1 "ARGUS DEVELOPMENTAL NEURO PND12 CNS MORPHOMETRICS";
57                       TITLE2 "PROC GLM - WITH TUKEYS";
58           ENDSAS;

NOTE: Means from the MEANS statement are not adjusted for other terms in the model.  For adjusted means, use the LSMEANS statement.
NOTE: The PROCEDURE GLM printed pages 5-25.

NOTE: SAS Institute Inc., SAS Campus Drive, Cary, NC USA 27513-2414
```

1 PERCHLORATE NEURODEVELOPMENTAL ARGUS 1613-002 - RAW DATA 15:56 Tuesday, January 26, 1999 1

OBS	SEX	DOSE	RATNO	BRAINWT	CEREBRUM	APCBLM	FCORTEX	PCORTEX	CAUDPUT	CORPCOL	HIPPO	CEREBLL	XGEM	TRT
1	F	1	2122	1.233	12.6	3.0	1224	1344	2208	192	912	3120	43	1--------CONTROL
2	F	1	2136	1.365	12.8	3.5	1512	1440	2448	259	1056	3696	36	1--------CONTROL
3	F	1	2170	1.342	12.9	3.0	1584	1512	2304	288	1104	3600	41	1--------CONTROL
4	F	1	2172	1.517	13.5	3.0	1632	1536	2496	298	1128	3984	41	1--------CONTROL
5	F	1	2185	1.321	12.5	3.2	1416	1296	2208	269	1152	3552	48	1--------CONTROL
6	F	1	2194	1.280	12.5	2.9	1536	1488	2304	336	960	3552	41	1--------CONTROL
7	F	2	2132	1.259	12.6	4.0	1440	1392	2304	259	984	3360	48	2--0.1_mg/kg/day
8	F	2	2133	1.168	12.3	3.7	1440	1392	2160	269	840	3072	46	2--0.1_mg/kg/day
9	F	2	2145	1.419	13.2	3.3	1560	1656	2256	288	1008	3840	41	2--0.1_mg/kg/day
10	F	2	2151	1.212	12.8	3.5	1488	1416	2016	269	1080	3456	41	2--0.1_mg/kg/day
11	F	2	2165	1.222	12.5	3.3	1488	1488	2064	259	912	3360	41	2--0.1_mg/kg/day
12	F	2	2174	1.347	13.2	4.1	1440	1392	2160	250	960	3696	43	2--0.1_mg/kg/day
13	F	3	2123	1.278	12.4	3.4	1344	1392	2304	307	1080	3024	41	3--1.0_mg/kg/day
14	F	3	2124	1.310	12.9	3.0	1296	1440	2400	336	1032	3552	36	3--1.0_mg/kg/day
15	F	3	2140	1.182	12.6	3.0	1464	1464	2352	355	1056	3264	36	3--1.0_mg/kg/day
16	F	3	2143	1.254	12.9	3.0	2198	1440	2448	346	1008	3168	36	3--1.0_mg/kg/day
17	F	3	2193	1.314	12.6	2.9	1392	1512	2256	355	936	3696	41	3--1.0_mg/kg/day
18	F	3	2198	1.330	13.2	3.3	1632	1608	2352	326	1008	3504	41	3--1.0_mg/kg/day
19	M	1	2002	1.375	13.2	3.4	1440	1416	2592	278	1080	3888	41	1--------CONTROL
20	M	1	2008	1.213	12.7	3.2	1296	1344	2400	240	1056	3648	36	1--------CONTROL
21	M	1	2036	1.357	12.7	3.2	1224	1368	2640	336	1248	3552	36	1--------CONTROL
22	M	1	2062	1.252	12.5	2.9	1368	1368	2352	240	936	3168	41	1--------CONTROL
23	M	1	2067	1.389	13.0	3.4	1368	1392	2544	384	1080	3696	41	1--------CONTROL
24	M	1	2094	1.335	13.2	3.1	1560	1632	2400	336	1080	3216	36	1--------CONTROL
25	M	2	2001	1.335	13.0	3.5	1464	1440	2400	365	984	3456	41	2--0.1_mg/kg/day
26	M	2	2019	1.289	13.0	3.5	1440	1440	2496	307	912	3312	36	2--0.1_mg/kg/day
27	M	2	2026	1.240	13.1	3.1	1392	1368	2304	259	888	3360	34	2--0.1_mg/kg/day
28	M	2	2039	1.250	13.1	3.8	1512	1488	2304	307	912	3312	31	2--0.1_mg/kg/day
29	M	2	2076	1.267	12.6	4.0	1272	1464	2016	240	864	3216	24	2--0.1_mg/kg/day
30	M	2	2097	1.208	12.3	3.0	1464	1464	2304	269	888	3264	43	2--0.1_mg/kg/day
31	M	3	2010	1.356	13.0	3.2	1608	1584	2640	528	1152	3504	36	3--1.0_mg/kg/day
32	M	3	2020	1.194	13.0	3.0	1584	1464	2688	317	984	3168	41	3--1.0_mg/kg/day
33	M	3	2028	1.249	12.7	2.2	1080	1296	2544	557	1200	3120	36	3--1.0_mg/kg/day
34	M	3	2037	1.353	13.0	3.5	1344	1512	2400	307	1032	3792	36	3--1.0_mg/kg/day
35	M	3	2041	1.289	13.0	3.2	1080	1440	2304	298	1104	3216	41	3--1.0_mg/kg/day
36	M	3	2043	1.321	13.0	2.9	1080	1488	2448	365	1296	3744	41	3--1.0_mg/kg/day

GROUP MEANS BY TREATMENT

TRT=1--------CONTROL

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	12	1.3315833	0.0237300	1.2130000	1.5170000	0.0822031	0.0067574	6.1733379
CEREBRUM	12	12.8416667	0.0941134	12.5000000	13.5000000	0.3260182	0.1062879	2.5387532
APCBLM	12	3.1500000	0.0583874	2.9000000	3.5000000	0.2022600	0.0409091	6.4209511
FCORTEX	12	1430.00	39.9044313	1224.00	1632.00	138.2330049	19108.36	9.6666437
PCORTEX	12	1428.00	28.1037041	1296.00	1632.00	97.3540866	9477.82	6.8175131
CAUDPUT	12	2408.00	40.8634088	2208.00	2640.00	141.5550006	20037.82	5.8785299
CORPCOL	12	288.0000000	15.4120181	192.0000000	384.0000000	53.3887969	2850.36	18.5377767
HIPPO	12	1066.00	27.3096719	912.0000000	1248.00	94.6034787	8949.82	8.8746228
CEREBLL	12	3556.00	77.8156329	3120.00	3984.00	269.5612597	72663.27	7.5804629
XGEM	12	40.0833333	1.0405297	36.0000000	48.0000000	3.6045006	12.9924242	8.9925170

TRT=2--0.1_mg/kg/day

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	12	1.2680000	0.0202286	1.1680000	1.4190000	0.0700740	0.0049104	5.5263397
CEREBRUM	12	12.8083333	0.0972799	12.3000000	13.2000000	0.3369875	0.1135606	2.6310023
APCBLM	12	3.5666667	0.1039619	3.0000000	4.1000000	0.3601347	0.1296970	10.0972333
FCORTEX	12	1450.00	20.3514574	1272.00	1560.00	70.4995164	4970.18	4.8620356
PCORTEX	12	1450.00	22.0000000	1368.00	1656.00	76.2102355	5808.00	5.2558783
CAUDPUT	12	2232.00	43.6181780	2016.00	2496.00	151.0978010	22830.55	6.7696147
CORPCOL	12	278.4166667	9.8868280	240.0000000	365.0000000	34.2489770	1172.99	12.3013386
HIPPO	12	936.0000000	19.8173478	840.0000000	1080.00	68.6493064	4712.73	7.3343276
CEREBLL	12	3392.00	59.4765041	3072.00	3840.00	206.0326541	42449.45	6.0740759
XGEM	12	39.0833333	1.9480306	24.0000000	48.0000000	6.7481760	45.5378788	17.2661219

TRT=3--1.0_mg/kg/day

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	12	1.2858333	0.0164353	1.1820000	1.3560000	0.0569335	0.0032414	4.4277517
CEREBRUM	12	12.8583333	0.0668086	12.4000000	13.2000000	0.2314316	0.0535606	1.7998572
APCBLM	12	3.0500000	0.0957427	2.2000000	3.5000000	0.3316625	0.1100000	10.8741796
FCORTEX	12	1425.17	90.8126471	1080.00	2198.00	314.5842374	98963.24	22.0735051
PCORTEX	12	1470.00	23.8403783	1296.00	1608.00	82.5854929	6820.36	5.6180607
CAUDPUT	12	2428.00	38.9498512	2256.00	2688.00	134.9262425	18205.09	5.5570940
CORPCOL	12	366.4166667	24.5956569	298.0000000	557.0000000	85.2018548	7259.36	23.2527236
HIPPO	12	1074.00	29.1141952	936.0000000	1296.00	100.8545307	10171.64	9.3905522
CEREBLL	12	3396.00	77.0643179	3024.00	3792.00	266.9586281	71266.91	7.8609726
XGEM	12	38.5000000	0.7537784	36.0000000	41.0000000	2.6111648	6.8181818	6.7822463

GROUP MEANS BY GENDER AND TREATMENT

TRT=1--------CONTROL SEX=F

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.3430000	0.0397131	1.2330000	1.5170000	0.0972769	0.0094628	7.2432557
CEREBRUM	6	12.8000000	0.1549193	12.5000000	13.5000000	0.3794733	0.1440000	2.9646353
APCBLM	6	3.1000000	0.0894427	2.9000000	3.5000000	0.2190890	0.0480000	7.0673878
FCORTEX	6	1484.00	59.8932383	1224.00	1632.00	146.7078730	21523.20	9.8859753
PCORTEX	6	1436.00	39.3954312	1296.00	1536.00	96.4987047	9312.00	6.7199655
CAUDPUT	6	2328.00	49.1853637	2208.00	2496.00	120.4790438	14515.20	5.1752167
CORPCOL	6	273.6666667	19.6547987	192.0000000	336.0000000	48.1442278	2317.87	17.5922879
HIPPO	6	1052.00	39.3954312	912.0000000	1152.00	96.4987047	9312.00	9.1728807
CEREBLL	6	3584.00	114.0385900	3120.00	3984.00	279.3363564	78028.80	7.7939832
XGEM	6	41.6666667	1.5846486	36.0000000	48.0000000	3.8815804	15.0666667	9.3157930

---------- TRT=1--------CONTROL SEX=M ----------

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.3201667	0.0291278	1.2130000	1.3890000	0.0713482	0.0050906	5.4044848
CEREBRUM	6	12.8833333	0.1194897	12.5000000	13.2000000	0.2926887	0.0856667	2.2718397
APCBLM	6	3.2000000	0.0774597	2.9000000	3.4000000	0.1897367	0.0360000	5.9292706
FCORTEX	6	1376.00	47.4636703	1224.00	1560.00	116.2617736	13516.80	8.4492568
PCORTEX	6	1420.00	43.5614508	1344.00	1632.00	106.7033270	11385.60	7.5143188
CAUDPUT	6	2488.00	48.6621002	2352.00	2640.00	119.1973154	14208.00	4.7908889
CORPCOL	6	302.3333333	24.0134222	240.0000000	384.0000000	58.8206313	3459.87	19.4555561
HIPPO	6	1080.00	40.6349603	936.0000000	1248.00	99.5349185	9907.20	9.2161962
CEREBLL	6	3528.00	115.4330975	3168.00	3888.00	282.7521883	79948.80	8.0145178
XGEM	6	38.5000000	1.1180340	36.0000000	41.0000000	2.7386128	7.5000000	7.1132800

---------- TRT=2--0.1_mg/kg/day SEX=F ----------

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.2711667	0.0384339	1.1680000	1.4190000	0.0941433	0.0088630	7.4060572
CEREBRUM	6	12.7666667	0.1520234	12.3000000	13.2000000	0.3723797	0.1386667	2.9168125
APCBLM	6	3.6500000	0.1408309	3.3000000	4.1000000	0.3449638	0.1190000	9.4510621
FCORTEX	6	1476.00	19.3494186	1440.00	1560.00	47.3962024	2246.40	3.2111248
PCORTEX	6	1456.00	42.7831743	1392.00	1656.00	104.7969465	10982.40	7.1975925
CAUDPUT	6	2160.00	44.6855681	2016.00	2304.00	109.4568408	11980.80	5.0674463
CORPCOL	6	265.6666667	5.3395797	250.0000000	288.0000000	13.0792456	171.0666667	4.9231790
HIPPO	6	964.0000000	33.6095225	840.0000000	1080.00	82.3261805	6777.60	8.5400602
CEREBLL	6	3464.00	111.1395519	3072.00	3840.00	272.2351924	74112.00	7.8589836
XGEM	6	43.3333333	1.2292726	41.0000000	48.0000000	3.0110906	9.0666667	6.9486706

GROUP MEANS BY GENDER AND TREATMENT

---------- TRT=2--0.1_mg/kg/day SEX=M ----------

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV

BRAINWT	6	1.2648333	0.0178688	1.2080000	1.3350000	0.0437695	0.0019158	3.4604932
CEREBRUM	6	12.8500000	0.1335415	12.3000000	13.1000000	0.3271085	0.1070000	2.5455918
APCBLM	6	3.4833333	0.1579381	3.0000000	4.0000000	0.3868678	0.1496667	11.1062516
FCORTEX	6	1424.00	34.3161769	1272.00	1512.00	84.0571234	7065.60	5.9028879
PCORTEX	6	1444.00	16.8760185	1368.00	1488.00	41.3376342	1708.80	2.8627170
CAUDPUT	6	2304.00	65.5804849	2016.00	2496.00	160.6387251	25804.80	6.9721669
CORPCOL	6	291.1666667	18.3456020	240.0000000	365.0000000	44.9373638	2019.37	15.4335537
HIPPO	6	908.0000000	16.8760185	864.0000000	984.0000000	41.3376342	1708.80	4.5526029
CEREBLL	6	3320.00	33.7520370	3216.00	3456.00	82.6752684	6835.20	2.4902189
XGEM	6	34.8333333	2.8215441	24.0000000	43.0000000	6.9113433	47.7666667	19.8411770

---------- TRT=3--1.0_mg/kg/day SEX=F ----------

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.2780000	0.0222231	1.1820000	1.3300000	0.0544353	0.0029632	4.2594118
CEREBRUM	6	12.7666667	0.1173788	12.4000000	13.2000000	0.2875181	0.0826667	2.2521001
APCBLM	6	3.1000000	0.0816497	2.9000000	3.4000000	0.2000000	0.0400000	6.4516129
FCORTEX	6	1554.33	137.3350324	1296.00	2198.00	336.4007531	113165.47	21.6427677
PCORTEX	6	1476.00	30.8285582	1392.00	1608.00	75.5142371	5702.40	5.1161407
CAUDPUT	6	2352.00	27.7128129	2256.00	2448.00	67.8822510	4608.00	2.8861501
CORPCOL	6	337.5000000	7.6365350	307.0000000	355.0000000	18.7056141	349.9000000	5.5424042
HIPPO	6	1020.00	20.3174802	936.0000000	1080.00	49.7674592	2476.80	4.8791627
CEREBLL	6	3368.00	104.7358582	3024.00	3696.00	256.5494104	65817.60	7.6172628
XGEM	6	38.5000000	1.1180340	36.0000000	41.0000000	2.7386128	7.5000000	7.1132800

---------- TRT=3--1.0_mg/kg/day SEX=M ----------

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.2936667	0.0258865	1.1940000	1.3560000	0.0634087	0.0040207	4.9014734
CEREBRUM	6	12.9500000	0.0500000	12.7000000	13.0000000	0.1224745	0.0150000	0.9457489
APCBLM	6	3.0000000	0.1807392	2.2000000	3.5000000	0.4427189	0.1960000	14.7572957
FCORTEX	6	1296.00	103.6918512	1080.00	1608.00	253.9921259	64512.00	19.5981579
PCORTEX	6	1464.00	39.1918359	1296.00	1584.00	96.0000000	9216.00	6.5573770
CAUDPUT	6	2504.00	59.9733274	2304.00	2688.00	146.9040503	21580.80	5.8667752
CORPCOL	6	395.3333333	47.6337882	298.0000000	557.0000000	116.6784756	13613.87	29.5139483
HIPPO	6	1128.00	46.3724056	984.0000000	1296.00	113.5887318	12902.40	10.0699230
CEREBLL	6	3424.00	121.8523697	3120.00	3792.00	298.4761297	89088.00	8.7171767
XGEM	6	38.5000000	1.1180340	36.0000000	41.0000000	2.7386128	7.5000000	7.1132800

PROC GLM - WITH TUKEYS

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	3	1--------CONTROL 2--0.1_mg/kg/day 3--1.0_mg/kg/day
SEX	2	F M

Number of observations in data set = 36

General Linear Models Procedure

Dependent Variable: BRAINWT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.02823647	0.00564729	1.05	0.4079
Error	30	0.16157983	0.00538599		
Corrected Total	35	0.18981631			

R-Square	C.V.	Root MSE	BRAINWT Mean
0.148757	5.666522	0.07338933	1.29513889

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	0.02581572	0.01290786	2.40	0.1082
SEX	1	0.00018225	0.00018225	0.03	0.8553
TRT*SEX	2	0.00223850	0.00111925	0.21	0.8135

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.02581572	0.01290786	2.40	0.1082
SEX	1	0.00018225	0.00018225	0.03	0.8553
TRT*SEX	2	0.00223850	0.00111925	0.21	0.8135

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PROC GLM - WITH TUKEYS

General Linear Models Procedure

Dependent Variable: CEREBRUM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.15805556	0.03161111	0.33	0.8902
Error	30	2.86500000	0.09550000		
Corrected Total	35	3.02305556			

R-Square	C.V.	Root MSE	CEREBRUM Mean
0.052283	2.407511	0.30903074	12.83611111

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	0.01555556	0.00777778	0.08	0.9220
SEX	1	0.12250000	0.12250000	1.28	0.2664
TRT*SEX	2	0.02000000	0.01000000	0.10	0.9009

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.01555556	0.00777778	0.08	0.9220
SEX	1	0.12250000	0.12250000	1.28	0.2664
TRT*SEX	2	0.02000000	0.01000000	0.10	0.9009

General Linear Models Procedure

Dependent Variable: APCBLM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1.94555556	0.38911111	3.97	0.0070
Error	30	2.94333333	0.09811111		
Corrected Total	35	4.88888889			

R-Square	C.V.	Root MSE	APCBLM Mean
0.397955	9.621305	0.31322693	3.25555556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	1.80222222	0.90111111	9.18	0.0008
SEX	1	0.02777778	0.02777778	0.28	0.5986
TRT*SEX	2	0.11555556	0.05777778	0.59	0.5612

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	1.80222222	0.90111111	9.18	0.0008
SEX	1	0.02777778	0.02777778	0.28	0.5986
TRT*SEX	2	0.11555556	0.05777778	0.59	0.5612

General Linear Models Procedure

Dependent Variable: FCORTEX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	247472.55555554	49494.51111111	1.34	0.2756
Error	30	1110147.33333334	37004.91111111		
Corrected Total	35	1357619.88888888			

R-Square	C.V.	Root MSE	FCORTEX Mean
0.182284	13.40482	192.36660602	1435.05555556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	4160.22222222	2080.11111111	0.06	0.9454
SEX	1	175002.77777778	175002.77777778	4.73	0.0377
TRT*SEX	2	68309.55555556	34154.77777778	0.92	0.4083

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	4160.22222222	2080.11111111	0.06	0.9454
SEX	1	175002.77777778	175002.77777778	4.73	0.0377
TRT*SEX	2	68309.55555556	34154.77777778	0.92	0.4083

General Linear Models Procedure

Dependent Variable: PCORTEX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	12224.00000000	2444.80000000	0.30	0.9068
Error	30	241536.00000000	8051.20000000		
Corrected Total	35	253760.00000000			

R-Square	C.V.	Root MSE	PCORTEX Mean
0.048172	6.191017	89.72847931	1449.33333333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	10592.00000000	5296.00000000	0.66	0.5253
SEX	1	1600.00000000	1600.00000000	0.20	0.6590
TRT*SEX	2	32.00000000	16.00000000	0.00	0.9980

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	10592.00000000	5296.00000000	0.66	0.5253
SEX	1	1600.00000000	1600.00000000	0.20	0.6590
TRT*SEX	2	32.00000000	16.00000000	0.00	0.9980

General Linear Models Procedure

Dependent Variable: CAUDPUT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	487488.00000000	97497.60000000	6.31	0.0004
Error	30	463488.00000000	15449.60000000		
Corrected Total	35	950976.00000000			

R-Square	C.V.	Root MSE	CAUDPUT Mean
0.512619	5.275739	124.29641990	2356.00000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	279168.00000000	139584.00000000	9.03	0.0008
SEX	1	207936.00000000	207936.00000000	13.46	0.0009
TRT*SEX	2	384.00000000	192.00000000	0.01	0.9877

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	279168.00000000	139584.00000000	9.03	0.0008
SEX	1	207936.00000000	207936.00000000	13.46	0.0009
TRT*SEX	2	384.00000000	192.00000000	0.01	0.9877

General Linear Models Procedure

Dependent Variable: CORPCOL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	70390.22222222	14078.04444444	3.85	0.0082
Error	30	109659.66666667	3655.32222222		
Corrected Total	35	180049.88888889			

R-Square	C.V.	Root MSE	CORPCOL Mean
0.390948	19.44375	60.45926085	310.94444444

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	55940.05555556	27970.02777778	7.65	0.0021
SEX	1	12544.00000000	12544.00000000	3.43	0.0738
TRT*SEX	2	1906.16666667	953.08333333	0.26	0.7722

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	55940.05555556	27970.02777778	7.65	0.0021
SEX	1	12544.00000000	12544.00000000	3.43	0.0738
TRT*SEX	2	1906.16666667	953.08333333	0.26	0.7722

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General Linear Models Procedure

Dependent Variable: HIPPO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	190784.00000000	38156.80000000	5.31	0.0013
Error	30	215424.00000000	7180.80000000		
Corrected Total	35	406208.00000000			

R-Square	C.V.	Root MSE	HIPPO Mean
0.469671	8.264590	84.73960113	1025.33333333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	144032.00000000	72016.00000000	10.03	0.0005
SEX	1	6400.00000000	6400.00000000	0.89	0.3527
TRT*SEX	2	40352.00000000	20176.00000000	2.81	0.0761

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	144032.00000000	72016.00000000	10.03	0.0005
SEX	1	6400.00000000	6400.00000000	0.89	0.3527
TRT*SEX	2	40352.00000000	20176.00000000	2.81	0.0761

General Linear Models Procedure

Dependent Variable: CEREBLL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	291072.00000000	58214.40000000	0.89	0.5021
Error	30	1969152.00000000	65638.40000000		
Corrected Total	35	2260224.00000000			

R-Square	C.V.	Root MSE	CEREBLL Mean
0.128780	7.430392	256.19992194	3448.00000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	210048.00000000	105024.00000000	1.60	0.2186
SEX	1	20736.00000000	20736.00000000	0.32	0.5783
TRT*SEX	2	60288.00000000	30144.00000000	0.46	0.6361

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	210048.00000000	105024.00000000	1.60	0.2186
SEX	1	20736.00000000	20736.00000000	0.32	0.5783
TRT*SEX	2	60288.00000000	30144.00000000	0.46	0.6361

General Linear Models Procedure

Dependent Variable: XGEM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	262.22222222	52.44444444	3.33	0.0164
Error	30	472.00000000	15.73333333		
Corrected Total	35	734.22222222			

R-Square	C.V.	Root MSE	XGEM Mean
0.357143	10.11296	3.96652661	39.22222222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	15.38888889	7.69444444	0.49	0.6180
SEX	1	136.11111111	136.11111111	8.65	0.0062
TRT*SEX	2	110.72222222	55.36111111	3.52	0.0424

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	15.38888889	7.69444444	0.49	0.6180
SEX	1	136.11111111	136.11111111	8.65	0.0062
TRT*SEX	2	110.72222222	55.36111111	3.52	0.0424

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: BRAINWT

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 0.005386
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 0.0739

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1.33158	12	1--------CONTROL
A			
A	1.28583	12	3--1.0_mg/kg/day
A			
A	1.26800	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: CEREBRUM

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 0.0955
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 0.311

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	12.8583	12	3--1.0_mg/kg/day
A			
A	12.8417	12	1--------CONTROL
A			
A	12.8083	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: APCBLM

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 0.098111
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 0.3153

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	3.5667	12	2--0.1_mg/kg/day
B	3.1500	12	1--------CONTROL
B			
B	3.0500	12	3--1.0_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: FCORTEX

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 37004.91
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 193.61

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1450.00	12	2--0.1_mg/kg/day
A			
A	1430.00	12	1--------CONTROL
A			
A	1425.17	12	3--1.0_mg/kg/day

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General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: PCORTEX

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 8051.2
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 90.309

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1470.00	12	3--1.0_mg/kg/day
A			
A	1450.00	12	2--0.1_mg/kg/day
A			
A	1428.00	12	1--------CONTROL

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General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: CAUDPUT

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 15449.6
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 125.1

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	2428.00	12	3--1.0_mg/kg/day
A			
A	2408.00	12	1--------CONTROL
B	2232.00	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: CORPCOL

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 3655.322
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 60.85

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	366.42	12	3--1.0_mg/kg/day
B	288.00	12	1--------CONTROL
B			
B	278.42	12	2--0.1_mg/kg/day

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General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: HIPPO

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 7180.8
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 85.288

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1074.00	12	3--1.0_mg/kg/day
A			
A	1066.00	12	1---------CONTROL
B	936.00	12	2--0.1_mg/kg/day

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PROC GLM - WITH TUKEYS

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: CEREBLL

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 65638.4
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 257.86

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	3556.0	12	1--------CONTROL
A			
A	3396.0	12	3--1.0_mg/kg/day
A			
A	3392.0	12	2--0.1_mg/kg/day

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General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: XGEM

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 15.73333
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 3.9922

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	40.083	12	1--------CONTROL
A			
A	39.083	12	2--0.1_mg/kg/day
A			
A	38.500	12	3--1.0_mg/kg/day

February 1, 1999 EPA Assessment Submission

Attachment #3
Nonparametric Reanalysis of Thyroid Histopathology in Pups on PND5 from Argus (1998a) Neurodevelopmental Study

A. EPA analysis (Marcus, 1999)

ATTENTION PANEL MEMBER(S):

JOE HASEMAN
SUSAN PORTERFIELD
TOM ZOELLER

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
RESEARCH TRIANGLE PARK, NC 27711

February 1, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Statistical Analyses of Standard Histopathological Measures of Thyroid Hypertrophy and Follicular Lumen Size Decrease in PND5 Rats

FROM: Allan H. Marcus, EMAG/NCEA-RTP (MD-52) Allan H Marcus

TO: Annie Jarabek, HPAG/NCEA-RTP (MD-52)

Attached is a set of statistical analyses of the histology data, provided as severity scores for both histology measures individual animals, that I received from you as a telefax from WPAFB (AFRL/HESD). A copy of these data is appended to the memo. I have corrected some errors in a draft version of 12/29/98, in response to specific comments from Dr. Joseph Haseman regarding the number of animals used in the analyses, identification of sub-groups, and expanding the methods of analysis to exact significance levels appropriate to these small sample sizes.

The raw data in the fax was converted into SYSTAT or StatXact data sets for further analyses. I can also export the data to spreadsheets or to SAS data sets, if needed. They are shown as Tables 1 to 6 in the attached memo. The changes are: (1) Table1 (level 1 at 10 mg/kg-day) frequency = 5; (2) Table 3 (level 1 severity at 0.1 mg/kg-day dose) frequency = 6, (at 10 mg/kg-day) frequency = 7; Table 6 (level 0 at 3 mg/kg-day) frequency = 1, (level 1 at 10 mg/kg-day) frequency = 2; Table 9 (levels "1 + 2" at dose 0) frequency = 6. The reported analyses were done using correct counts.

The exact small-sample Jonckheere-Terpstra test for ordered categories was used in Tables 1-5, and new Tables 1A-6A. These tests were based on assuming ordered categories of both dose and severity, hence are one-tailed tests. Monte Carlo approximation to the P-value was calculated in Table 6. Exact Fisher tests for 2X2 tables using the likelihood ratio criterion were carried out in Tables 7-12.

I understand that these analyses are based on data in the Argus rat developmental neurotoxicology study (Argus, 1998a). The 2x2 contingency table tests of association are straightforward and described in most elementary statistics texts. The logistic regression analyses in this version of SYSTAT used the iteratively reweighted least squares approach to maximum likelihood estimation described on p. 622 of the SYSTAT v. 5.0 manual (1995). These are very simple approaches, easily understood by most non-specialists. Further analyses using categorical regression methods may also be informative.

The sample sizes are on the small side for testing hypotheses. For that reason, the findings of marginal or statistically significant associations in the contingency table tests at 0.1 and 1 mg/kg-day are worrying, given that the study has small power to detect real effects of only modest magnitude. The logistic regression models are consistent with a steeper dose-response function at low doses than at high doses. The evidence as a whole leans toward a significant response at doses as low as 0.1 to 1 mg/kg-day. A larger study to look at these lower dose ranges would seem to be justified.

Attachment

Statistical Analyses of Standard Histopathological Measures of Thyroid Hypertrophy and Follicular Lumen Size Decrease in PND5 Rats

Allan H. Marcus, Statistician
National Center for Environmental Assessment – RTP

1. DATA STRUCTURE AND PURPOSE OF THE ANALYSES

The purpose of the analyses was to provide an assessment of possible trends in toxicity data provided to me by Annie Jarabek, based on the rat neurodevelopmental study data for pups postnatal on day 5 (PND5), reported in (Argus, 1998a). There were two toxicity endpoints: (1) Follicular epithelial cell hypertrophy (denoted HYPER), and (2) decrease in follicular lumen size (denoted SIZE). Both were coded on a discrete scale of increasing seriousness, as 0, 1, 2 for HYPER and 0, 1, 2, 3 for SIZE. There were separate studies for females and for males, so SEX was also a discrete variable. Each set of experiments was done at 5 dose levels: control (0 mg/kg-day), 0.1, 1, 3, and 10 mg/kg-day. DOSE effects could be evaluated either as an ordered categorical scale or as a numeric scale. Including DOSE as an ordered categorical scale allowed use of contingency table methods, whereas use of DOSE or log(DOSE) as a numeric scale allowed use of logistic regression models. These provide different but complementary information about the relationship, using elementary analytical methods.

2. TESTING ASSOCIATION IN CATEGORICAL RESPONSE DATA

The individual rat data were combined into contingency tables and entered into the SYSTAT (1995) data analysis system. The basic data tables are shown below, along with the results for tests of association with DOSE in a table with r rows and c columns as shown. The first set of tests was done by exact small-sample Jonckheere-Terpstra tests (StatXact, 1998) for association in ordered categories (DOSE, severity) for each sex and for both sexes, for both endpoints. We use the following symbols for significance: * for $0.01<P<0.05$, ** for $0.001<P<0.01$, *** for $P<0.001$, and # for $0.05 < P < 0.10$. Because of the ordering assumed in both dimensions of the dose-severity relationship, all tests are one-tailed tests.

TABLE 1

HYPERTROPHY, FEMALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	4	1	1
0.1	3	2	1
1	1	2	3
3	3	2	1
10	0	5	1

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN FEMALES: 0.0811#
DF=8

TABLE 2

HYPERTROPHY, MALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	5	1	0
0.1	1	4	1
1	2	3	1
3	1	4	1
10	0	2	4

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN MALES: 0.0004*** DF=8

TABLE 3

HYPERTROPHY, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	9	2	1
0.1	4	6	2
1	3	5	4
3	4	6	2
10	0	7	5

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION: 0.0005***, DF=8

TABLE 4
SIZE, FEMALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, FEMALE / DOSE, mg/kg-day	LEVEL 0	1	2	3
0	2	3	1	0
0.1	1	3	2	0
1	1	4	1	0
3	1	1	2	2
10	0	2	3	1

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN FEMALES: 0.0110*, DF=12

TABLE 5
SIZE, MALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, MALE DOSE, mg/kg-day	LEVEL 0	1	2	3
0	4	1	1	0
0.1	1	3	2	0
1	1	1	4	0
3	0	2	4	0
10	0	0	3	3

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN MALES: 0.0001***, DF=12

TABLE 6
SIZE, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, ALL DOSE, mg/kg-day	LEVEL 0	1	2	3
0	6	4	2	0
0.1	2	6	4	0
1	2	5	5	0
3	1	3	6	2
10	0	2	6	4

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN ALL SEXES: 0.0000***, DF=12

We also repeated these tests for a much more focused assessment of controls vs. dose 0.1 mg/kg, using all levels of severity, but maintaining the ordering of alternatives in the exact small-sample Jonckheere-Terpstra tests. This is shown in Tables 1A-6A.

TABLE 1A

HYPERTROPHY, FEMALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	4	1	1
0.1	3	2	1

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN FEMALES: 0.4621
DF=2

TABLE 2A

HYPERTROPHY, MALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	5	1	0
0.1	1	4	1

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN MALES: 0.0325*
DF=2

TABLE 3A

HYPERTROPHY, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	9	2	1
0.1	4	6	2

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION: 0.0432*, DF=2

TABLE 4A
SIZE, FEMALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, FEMALE	LEVEL 0	1	2
DOSE, mg/kg-day			
0	2	3	1
0.1	1	3	2

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN FEMALES: 0.3528, DF=2

TABLE 5A
SIZE, MALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, MALE	LEVEL 0	1	2
DOSE, mg/kg-day			
0	4	1	1
0.1	1	3	2

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN MALES: 0.1050, DF=2

TABLE 6A
SIZE, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, ALL DOSE, mg/kg-day	LEVEL 0	1	2
0	6	4	2
0.1	2	6	4

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN BOTH SEXES: 0.0661#, DF=2

Exact Fisher tests were performed on reduced 2 by 2 tables, using DOSE level 0.1 and 1 mg/kg-day vs. controls to see if there was a significant difference at low doses. Tests of the controls against the highest 2 doses were significant and are not shown here. The low-dose tests for HYPER used a combined HYPER score of 1+2 to combine the more serious effects These tables were then combined into single tables for the purpose of providing a concise display of the results. All of the tests are one-tailed likelihood ratio tests, following a natural ordering of alternatives.

TABLE 7
2 BY 2 CONTINENCY TABLE TESTS FOR HYPERTROPHY AT DOSE 0.1 mg/kg-day

SEX	FEMALE		MALE		ALL	
HYPER LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	4	2	5	1	9	3
DOSE 0.1	3	3	1	5	4	8
P VALUE	0.5000		0.0400*		0.0498*	

TABLE 8
2 BY 2 CONTINENCY TABLE TESTS FOR HYPERTROPHY AT DOSE 1 mg/kg-day

SEX	FEMALE		MALE		ALL	
HYPER LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	4	2	5	1	9	3
DOSE 1.0	1	5	2	4	3	9
P VALUE	0.1212		0.1212		0.0196*	

The 2 by 2 tests for SIZE effects required a more detailed level of the aggregated SIZE categories. We show separate results for category 0 vs. 1+2, and categories 0+1 vs. 2. Category 3 had no counts at dose levels 0, 0.1 and 1.

TABLE 9
2 BY 2 CONTINENCY TABLE TESTS FOR SIZE EFFECT AT DOSE 0.1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	2	4	4	2	6	6
DOSE 0.1	1	5	1	5	2	10
P VALUE	0.1212		0.1212		0.0965#	

TABLE 10
2 BY 2 CONTINENCY TABLE TESTS FOR SIZE AT DOSE 0.1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0+1	2	0+1	2	0+1	2
DOSE 0	5	1	5	1	10	2
DOSE 0.1	4	2	4	2	8	4
P VALUE	0.1212		0.1212		0.3202	

TABLE 11
2 BY 2 CONTINENCY TABLE TESTS FOR SIZE AT DOSE 1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	2	4	4	2	6	6
DOSE 1	1	5	1	5	2	10
P VALUE	0.1212		0.1212		0.0965#	

TABLE 12
2 BY 2 CONTINENCY TABLE TESTS FOR SIZE AT DOSE 1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0+1	2	0+1	2	0+1	2
DOSE 0	5	1	5	1	10	2
DOSE 1	5	1	2	4	7	5
P VALUE	0.5000		0.1212		0.1854	

3. LOGISTIC REGRESSION ANALYSIS

As a check on the overall relationship, we also carried out logistic regression analyses of response vs. dose and vs. log(dose), for males and females separately and for both sexes combined. The dose for controls was taken as 0, and log(dose) as log(0.01 mg/kg-day). The results are shown in the following tables.

TABLE 13
LOGISTIC REGRESSION COEFFICIENT OF HYPERTROPHY > 0 VS. DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.332	0.210	-16.90
MALE	0.614	0.397	-14.78
ALL	0.423*	0.192	-32.06

TABLE 14
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 0 VS. DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.335	0.313	-12.31
MALE	1.734	1.187	-10.68
ALL	0.614	0.378	-22.30

TABLE 15
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 1 VS. DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.198#	0.109	-18.34
MALE	0.635#	0.339	-15.15
ALL	0.279***	0.097	-35.66

TABLE 16
LOGISTIC REGRESSION COEFFICIENT OF HYPERTROPHY > 0 VS. LOG DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.342*	0.174	-17.08
MALE	0.532**	0.207	-13.95
ALL	0.426***	0.132	-31.49

TABLE 17
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 0 VS. LOG DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.269	0.205	-12.60
MALE	0.704**	0.284	-10.02
ALL	0.459***	0.166	-22.07

TABLE 18
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 1 VS. LOG DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.330#	0.179	-18.20
MALE	0.572**	0.208	-15.20
ALL	0.430***	0.132	-34.86

The relationship between non-transformed dose and hypertrophy is statistically significant in both sexes combined, and positive but not significant in both sexes separately. The relationship with the logarithm of dose is significant or very significant in all analyses. This suggests that the risk of a hypertrophic response increases as (roughly) the 0.3 to 0.5 power of dose. Since the dose-response function is nonlinear with a steeper slope near the origin, the possibility of significant responses at low doses is consistent with the contingency table tests.

The regression coefficients of any size > 0 vs. untransformed dose are positive but not significant, whereas after log-transformation, the effects for males and for both sexes are very significant. If the severity cutpoint for SIZE is taken as levels 2+3 vs. levels 0+1, then the relationship with dose is marginally significant in either sex and highly significant when sexes are combined. The effects for males and for both sexes combined are highly significant in the model for log of dose, which also suggests that the SIZE response probability at low doses increases as roughly the 0.3 to 0.5 power of dose.

Additional logistic regression models explored the possibility of a dose-sex interaction, with males having a steeper dose-response curve. No statistically significant gender effect was found, but it is unlikely that these small samples allow sufficient power to detect this effect.

4. SUMMARY

There appears to be strong evidence for a dose-response relationship between perchlorate dose and both endpoints, follicular epithelial cell hypertrophy and decrease in follicular lumen size. Even though the number of rats in each treatment group is smaller than is desirable to have substantial power against real

effects of modest size at the two lowest dose levels, attention should be paid to the simple comparisons in Tables 2A, 3A, 7 and 8, which suggest a significant increase in hypertrophy for males, and for both groups combined at both 0.1 and 1 mg/kg-day (significant). One should note that the differences lie in the expected direction if there is a real dose-response relationship. Although there may be a dose-sex interaction, with males showing stronger effects than females, this was not significant, and combining the sexes gave evidence for an effect on follicular epithelial cell hypertrophy.

Similar analyses did not find strongly significant decreases in follicular lumen cell size at the lowest two levels using the very basic contingency table tests in Tables 9 through12, nor in Tables 4A, 5A, and 6A. However, the logistic regression models suggested that there is a very significant dose response relationship overall, with a strong model-based suggestion of a steeper dose-response relationship for lumen cell size at lower doses.

Taking the small samples sizes and limited power of these data into account, there is an indication of increased effects at levels as low as 0.1 to 1 mg/kg-day, particularly for the follicular epithelial cell hypertrophy in males.

5. REFERENCES

1. Argus, 1998a. A neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to rats [report ammendment: July 27, 1998]. Argus Research Laboratories, Inc., Horsham, PA. Argus Protocol #1613-002,

2. Wilkinson, L. SYSTAT: The System for Statistics. SYSTAT Inc., Evanston, Il., 1995.

3. StatXact Program. Cytel Inc., Cambridge, MA. 1998.

Appendix: Data as received by telefax.

February 1, 1999 EPA Assessment Submission

Attachment #4
Hormone Data Analysis for F0 and F1
from Argus (1998b) 2-Generation Reproductive Study

A. EPA analysis (Geller, 1999b)
B. EPA analysis (House, 1999)

ATTENTION PANEL MEMBER(S):

TOM ZOELLER
JOE HASEMAN
SUSAN PORTERFIELD

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

Neurotoxicology Division, MD-74B

MEMORANDUM

Date: February 1, 1999

Subject: Analysis of the Thyroid Hormone Data from the Rat Two Generation Reproduction Study (Argus, 1998b)

From: for Andrew M. Geller
Neurotoxicology Division, MD-74B
National Health Effects and Environmental Research Laboratory

To: Annie Jarabek
National Center for Environmental Assessment

Attached is the statistical analysis of the hormone data from the Argus Rat Developmental Neurotoxicology Study (Argus, 1998b). A memo (York, 1999g) from Argus Laboratories (RE: Argus Protocol #1416-001, 20 November 1998) provided thyroid hormone and thyrotropin data from the oral (drinking water) two-generation reproductive study of ammonium perhclorate in the rat. Data were supplied on diskette in the form of ASCII text reports, one report for each gender/age group, and imported in ASCII form to SAS for further analysis. I have attached a description of how the analyses were done, a description of results, and summary graphs.

An alternative statistical analysis for the F1 generation, per suggestion by Joseph Haseman, is provided in the memo from Dennis House (1999) using these same data. These analyses have been provided for comparative purposes.

Analyses of Hormone Data from the Argus Oral (Drinking Water) Two-Generation Reproduction Study

Summary: A memo from Argus Laboratories (RE: Argus Protocol #1416-001, 20 November 1998) contains thyroid hormone and thyrotrophin data from the Oral (Drinking Water) Two-Generation reproduction Study of ammonium perchlorate in the rat. The following is a statistical analysis of the thyroid and pituitary hormone data (T4, thyroxine; T3, triiodothyronine; TSH, thyroid stimulating hormone) found in that report. At the time of this analysis, data were available from both the F0 generation, females and males sacrificed at 5 and 6 months of age, respectively, and the F1 generation, one male and one female from each litter, sacrificed on postnatal day 21 (PND21). Males were sacrificed after 13 weeks of exposure, i.e., approximately 91 days. Females were sacrificed after 16 weeks, i.e. at weaning, approximately 120 days of exposure.

Data from the F0 generation were re-analyzed to look for dose and gender effects. Data from the F1 generation were re-analyzed using gender as a repeated measure within each litter. Results of these re-analyses are similar to those stated in the memo from Argus RE: Protocol 1416-001 (20 November 1998).

For the F0 generation, a NOEL of 3.0 mg/kg/day was identified from a decrease in T4 and an increase in TSH of male rats. These results are consistent with the known mechanism-of-action (MOA) of perchlorate (inhibition of thyroid hormones). The increased TSH is likely a result of the activation of the pituitary-thyroid feedback mechanism. These data are not consistent with the results of the 90-day drinking water study (Springborn Laboratories, Inc., 1998). In that study, 90 days of exposure resulted in LOELs of 0.01 mg/kg/day for T3 and T4 and a NOEL of 0.05 mg/kg/day for TSH.

For the F1 generation, a LOAEL of 0.3 mg/kg/day was identified for a decrease in TSH level, inconsistent with known MOA of perchlorate. This data is inconsistent with results from the Neurodevelopmental Toxicity Study (Argus, 1998a, Crofton, 1998f). In the Neurodevelopmental study, dose-related decreases of T4 and T3 and dose-related increase of TSH were found. Possible reasons for this disparity are discussed.

Data: All data were supplied in the form of ASCII text reports, one report for each gender/age group. Data were exported as ASCII files for analyses by SAS.

F0 generation: Data for dependent measures (T4, T3 and TSH) were subjected to separate two-way ANOVAs. Treatment (dose) and sex were the independent between-subjects variables. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. Where there was a dose x sex interaction, separate one-way ANOVAs were run for each gender.

F1 generation: Data for dependent measures (T4, T3, TSH) were subjected to separate repeated-measures ANOVAs. Treatment (dose) was the independent between-subjects variable. Sex was a within-litter repeated-measures variable. The repeated-measures analysis requires a full set of data for each litter, i.e. 1 male and 1 female. Data was missing from 4 litters (1 male from each of 0, 0.3, and 30 mg/kg/day dose groups and 1 female from 30 mg/kg/day), reducing the sample size in the analysis from 99 to 95. Mean contrasts were performed using Tukey's

Studentized Range (HSD) Test.

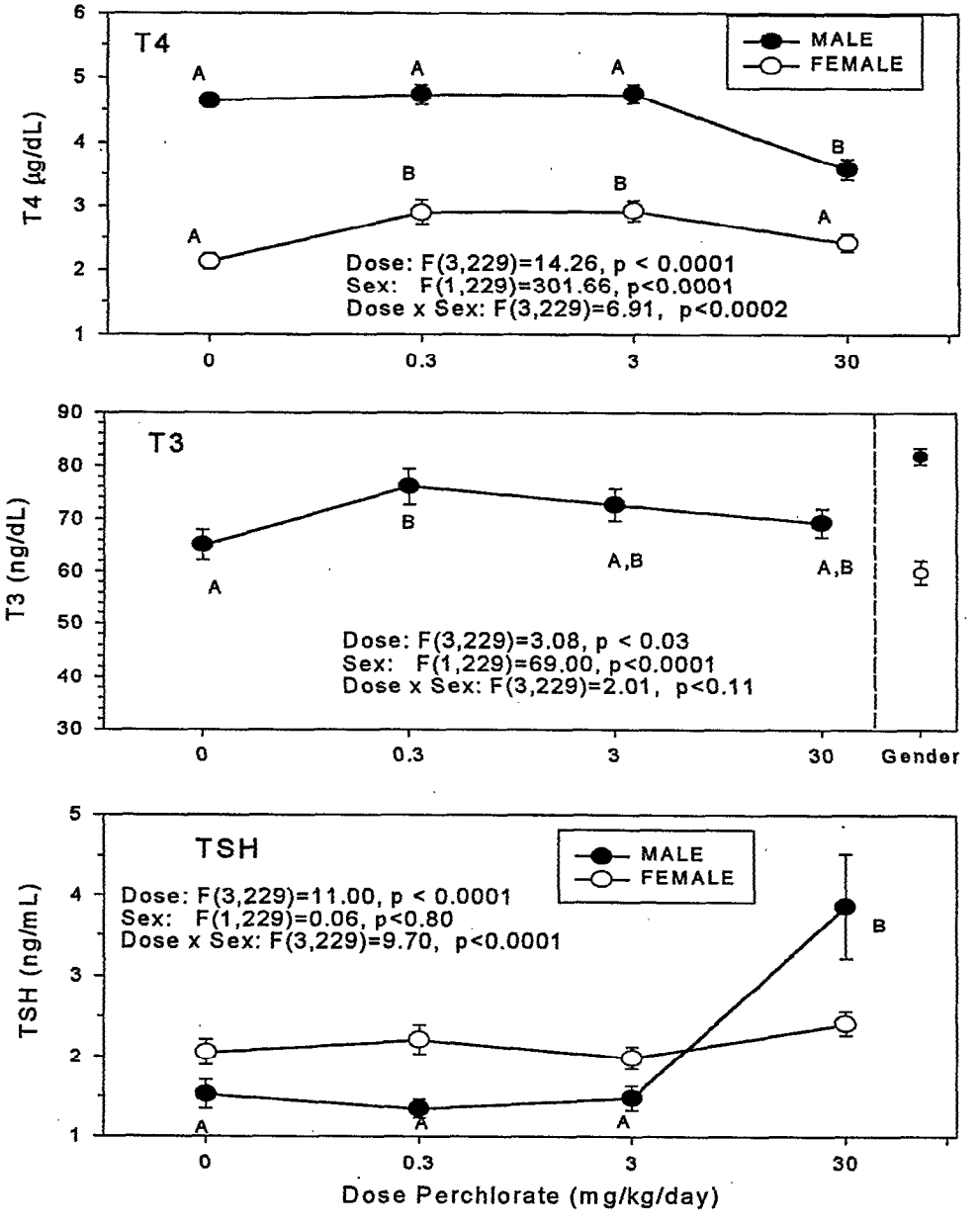
To correct for multiple comparisons (i.e., separate analyses for T4 and TSH) the acceptable alpha for significance (for all interaction main effects tests) was corrected to 0.029 (alpha of 0.05 divided by the square root of the number of ANOVAs). SAS analysis code and output are attached in Appendix 1.

Data Analysis - Results:

F0 Generation: There were significant dose effects for T4 and TSH, and dose x sex effects for T4 and TSH (Figure 1). Given our assumptions about the mechanism of action (MOA) of perchlorate (i.e., iodide uptake inhibition resulting in reduced levels of T4 and T3, and an increase in TSH), only the effects on T4 and TSH levels for males can be considered biologically significant. NOELs were identified for males only for T4 and TSH at a dose of 3.0 mg/kg/day. There were also significant effects of sex on T4 and T3 levels.

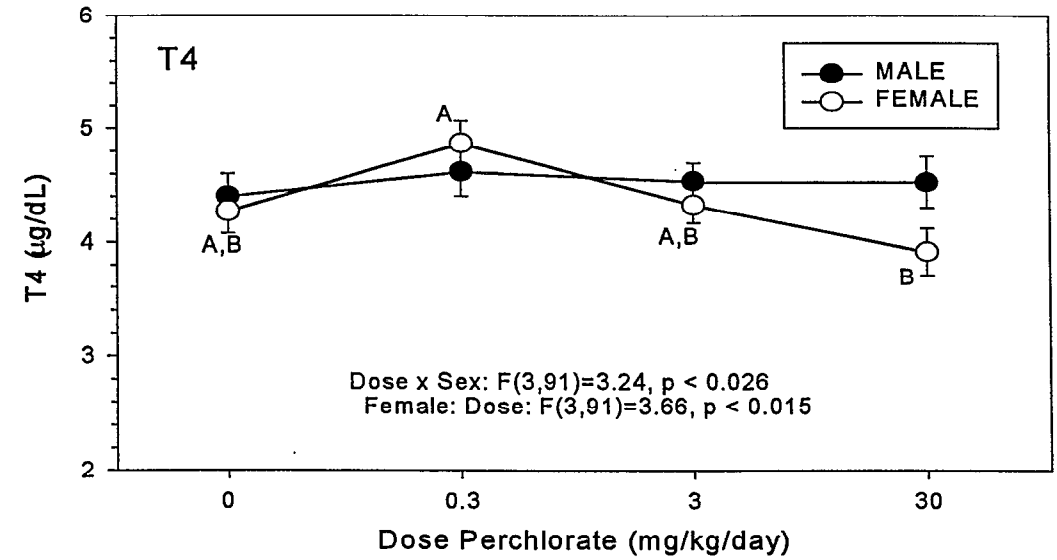
F1 Generation: There were no significant main effects of dose on T4, T3, or TSH. There were significant dose x sex interactions for T4 and TSH (Figure 2). The significant effect of dose on female T4 data is due to an elevated level in the 0.3 mg/kg/day group relative to the high dose group and is not consistent with the MOA of perchlorate. There was a LOEL of 0.3 mg/kg/day for a reduction in TSH level in males; this is not consistent with the known MOA of perchlorate.

These results are different from those in the F1 generation of the Neurodevelopmental Toxicity study (Argus, 1998a, Crofton, 1998f). In PND5 pups exposed through gestation and lactation, there were significant dose-related reductions in T4 and T3, and a significant dose-related increase in TSH. One possible source of this disparity is that the PND21 weanlings tested in the Two-Generation study likely received a reduced dose of the test compound through lactation (Fisher, 1998b) and the slow addition of drinking water to their diets. This may have allowed recovery from the hormone deficits due to gestational effects still visible in the younger pups.



Figure

1. Effects of oral perchlorate exposure on hormone levels in F0 generation. Serum total thyroxine (T4) (top): There were significant dose, sex, and dose x sex effects. Means with different letters (on each function) were significantly different ($p<0.05$). Serum total triiodothyronine (T3)(middle): There was a significant effects of sex and a borderline significant effect of dose. Plot to right of dotted line illustrates sex effect (males>females). Serum thyroid stimulation hormone (TSH) (bottom): There were significant effects of dose and dose x sex. Means with different letters were significantly different ($p<0.05$).



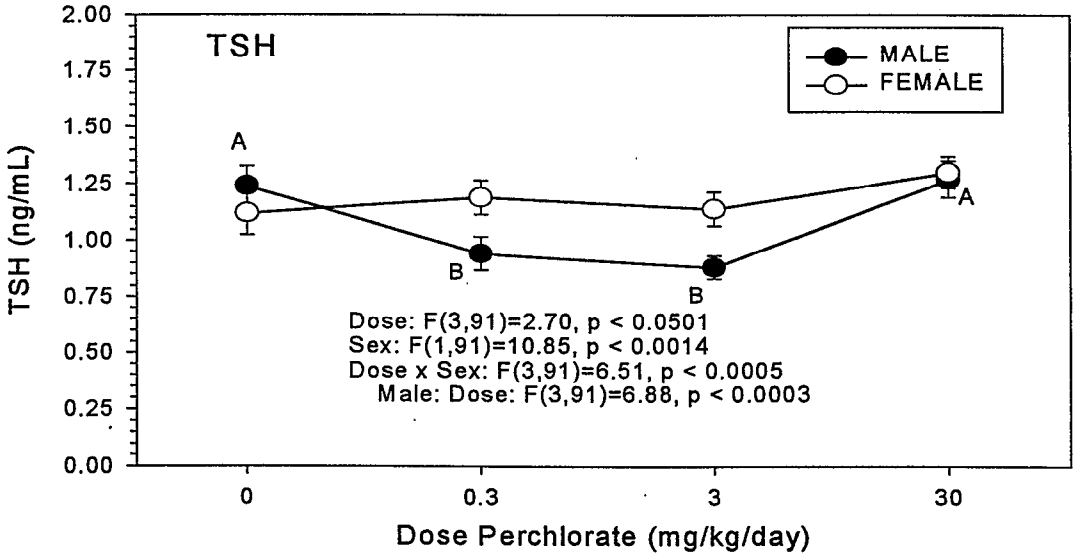


Figure 2. Effects of perchlorate exposure on hormone levels in F1 generation at post-natal day 21. Serum total thyroxine (T4) (top): There was a significant dose x sex effect, with a dose effect in females due to elevated T4 in the 0.3 mg/kg dose group. Means with different letters were significantly different ($p<0.05$). Serum thyroid stimulation hormone (TSH) (bottom): There were significant main effects of sex and dose x sex, with a dose effect in males. Means with different letters were significantly different ($p<0.05$).

APPENDIX 1 - Raw Data and Statistical Analysis

```
NOTE: Copyright (c) 1989-1996 by SAS Institute Inc., Cary, NC, USA.
NOTE: SAS (r) Proprietary Software Release 6.12  TS020
      Licensed to US ENVIRONMENTAL PROTECTION AGENCY, Site 0019614059.

NOTE: Running on ALPHASERVER Model 2100 5/300 Serial Number 80000000.


WARNING: Your system is scheduled to expire on February 18, 1999, which is 28 days from now. Please contact your installation
         representative to have your system renewed. The SAS system will no longer function on or after that date.
 Welcome to the NHEERL-RTP SAS Information Delivery System.


1          /* INPUT NEWLY RECEIVED THYROID HORMONE DATA FROM 2 GEN Reproduction STUDY.
2             DATA GENERATED BY ARGUS RESEARCH LABS, RECEIVED JAN 7, 1999 */
3
4          DATA F21; /* Female rats, generation F1,  day 21 */
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
5             INFILE '[GELLER.BMD]GEN2F21.DAT';
6             INPUT a $ no id grp sex $ age $ tsh1 t31 t41;
7             DROP a no sex;
8          RUN;

NOTE: The infile '[GELLER.BMD]GEN2F21.DAT' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]GEN2F21.DAT

NOTE: 98 records were read from the infile '[GELLER.BMD]GEN2F21.DAT'.
      The minimum record length was 74.
      The maximum record length was 74.
NOTE: The data set WORK.F21 has 98 observations and 6 variables.

9
10         DATA M21; /* Male rats, generation F1, day 21 */
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
11            INFILE '[GELLER.BMD]GEN2M21.DAT';
12            INPUT a $ no id grp sex $ age $ tsh2 t32 t42;
13            DROP a no sex;
14         RUN;

NOTE: The infile '[GELLER.BMD]GEN2M21.DAT' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]GEN2M21.DAT

NOTE: 96 records were read from the infile '[GELLER.BMD]GEN2M21.DAT'.
      The minimum record length was 74.
      The maximum record length was 74.
NOTE: The data set WORK.M21 has 96 observations and 6 variables.

15
16         PROC SORT DATA=f21;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
17            BY id;
18         RUN;

NOTE: The data set WORK.F21 has 98 observations and 6 variables.
```

```
 19
12                                    The SAS System                    09:23 Thursday, January 21, 1999

 20         PROC SORT DATA=m21;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
 21            BY id;
 22          RUN;

NOTE: The data set WORK.M21 has 96 observations and 6 variables.

 23
 24          DATA day21rep;
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
 25            MERGE f21 m21;
 26            BY id;
 27          RUN;

NOTE: The data set WORK.DAY21REP has 99 observations and 9 variables.

 28
 29          /*****************************************************************/
 30          /* For F0 generation, rats are not tracked by litter.  Therefore */
 31          /* simply concatenate the male and female data sets and analyze */
 32          /* with 2 way analysis of variance (grp, sex)                    */
 33          /*****************************************************************/
 34
 35          DATA F5M; /* Female rats, F0, 5 months */
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
 36            INFILE '[GELLER.BMD]GEN25MF.DAT';
 37            INPUT a $ no id grp sex $ age $ tsh t3 t4;
 38            DROP a no;
 39          RUN;

NOTE: The infile '[GELLER.BMD]GEN25MF.DAT' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]GEN25MF.DAT

NOTE: 119 records were read from the infile '[GELLER.BMD]GEN25MF.DAT'.
      The minimum record length was 74.
      The maximum record length was 74.
NOTE: The data set WORK.F5M has 119 observations and 7 variables.

 40
 41          DATA M6M; /* Male rats, F0, 6 months */
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
 42            INFILE '[GELLER.BMD]GEN26MM.DAT';
 43            INPUT a $ no id grp sex $ age $ tsh t3 t4;
 44            DROP a no;
 45          RUN;

NOTE: The infile '[GELLER.BMD]GEN26MM.DAT' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]GEN26MM.DAT

NOTE: 118 records were read from the infile '[GELLER.BMD]GEN26MM.DAT'.
      The minimum record length was 74.
      The maximum record length was 74.
```

```
NOTE: The data set WORK.M6M has 118 observations and 7 variables.

13                                                The SAS System                    09:23 Thursday, January 21, 1999

46
47          PROC SORT DATA=f5m;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
48            BY id;
49          RUN;

NOTE: The data set WORK.F5M has 119 observations and 7 variables.

50
51          PROC SORT DATA=m6m;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
52            BY id;
53          RUN;

NOTE: The data set WORK.M6M has 118 observations and 7 variables.

54
55          DATA F0;
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
56            set f5m m6m;
57          RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.

58
59
60          /*****************************************************************/
61          /*  Analysis of F1, by dose group with sex as a repeated measure      */
62          /*****************************************************************/
63          PROC SORT DATA=day21rep;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
64            BY grp;
65          RUN;

NOTE: The data set WORK.DAY21REP has 99 observations and 9 variables.

66
67          PROC PRINT;
WARNING: The BASE Product product with which PRINT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
68            TITLE1'Generation F1, DAY 21 data for repeated measures.';
69            TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
70          RUN;

NOTE: The PROCEDURE PRINT printed pages 1-2.

71
72          PROC MEANS N MEAN STDERR STD MIN MAX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
73            BY grp;
```

```
74          VAR tsh1 t31 t41 tsh2 t32 t42;
75          TITLE1'MEANS of Generation F1, DAY 21 data for repeated measures.';
76          TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
14                                                The SAS System                    09:23 Thursday, January 21, 1999

77        RUN;

NOTE: The PROCEDURE MEANS printed page 3.

78
79        PROC GLM DATA=day21rep;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
80          CLASS grp;
81          MODEL t41 t42=grp;
82          REPEATED sex 2 /SUMMARY;
83          MEANS grp /TUKEY LINES;
84          TITLE1'Generation F1,DAY 21, T4';
85          TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
86        RUN;

87

NOTE: The PROCEDURE GLM printed pages 4-12.

88        PROC GLM DATA=day21rep;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
89          CLASS grp;
90          model t31 t32=grp;
91          REPEATED sex 2 /SUMMARY;
92          MEANS grp /TUKEY LINES;
93          TITLE1'Generation F1,DAY 21, T3';
94          TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
95        RUN;

96

NOTE: The PROCEDURE GLM printed pages 13-21.

97        PROC GLM DATA=day21rep;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
98          CLASS grp;
99          model tsh1 tsh2=grp;
100         REPEATED sex 2 /SUMMARY;
101         MEANS grp /TUKEY LINES;
102         TITLE1'Generation F1,DAY 21, TSH';
103         TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
104       RUN;

105         /*************************************************/
106         /*    ANALYSIS OF F0, BY DOSE GRP AND SEX        */
107       /*************************************************/

NOTE: The PROCEDURE GLM printed pages 22-30.

108       PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
```

```
         representative to have it renewed.
109          BY grp sex;
110        RUN;

15                                                The SAS System                              09:23 Thursday, January 21, 1999

NOTE: The data set WORK.F0 has 237 observations and 7 variables.

111
112        PROC PRINT DATA= F0;
WARNING: The BASE Product product with which PRINT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
113          TITLE1'DATA FROM F0 GENERATION';
114        RUN;

NOTE: The PROCEDURE PRINT printed pages 31-35.

115
116        PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
117          BY sex;
118        RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.

119
120        PROC MEANS N MEAN STDERR STD MIN MAX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
121          BY sex;
122          TITLE'F0 Generation, Means by SEX';
123        RUN;

NOTE: The PROCEDURE MEANS printed page 36.

124
125        PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
126          BY grp;
127        RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.

128
129        PROC MEANS N MEAN STDERR STD MIN MAX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
130          BY grp;
131          TITLE'F0 Generation, Means by Dose Group';
132        RUN;

NOTE: The PROCEDURE MEANS printed page 37.

133
134        PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
```

```
135          BY grp sex;
136        RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.

16                                    The SAS System                    09:23 Thursday, January 21, 1999

137
138        PROC MEANS N MEAN STDERR STD MIN MAX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
139          BY grp sex;
140          TITLE'F0 Generation, Means by Dose and Sex';
141        RUN;

NOTE: The PROCEDURE MEANS printed pages 38-39.

142
143        PROC GLM DATA=F0;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
144          CLASSES grp sex;
145          model t4 t3 tsh=grp|sex;
146          MEANS grp|sex /TUKEY LINES;
147          TITLE'Generation F0, ADULT';
148        RUN;

NOTE: Means from the MEANS statement are not adjusted for other terms in the model.  For adjusted means, use the LSMEANS statement.
149

NOTE: The PROCEDURE GLM printed pages 40-49.

150        PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
151          BY sex;
152        RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.

153
154        PROC GLM DATA=F0;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
155          BY sex;
156          CLASSES grp;
157          MODEL t4 t3 tsh=grp;
158          MEANS grp/TUKEY LINES;
159          TITLE1'Generation F0, ADULT';
160          TITLE2'Analysis by Sex';
161        RUN;

NOTE: Interactivity disabled with BY processing.
162
NOTE: The PROCEDURE GLM printed pages 50-63.

NOTE: SAS Institute Inc., SAS Campus Drive, Cary, NC USA 27513-2414
```

1

Generation F1, DAY 21 data for repeated measures. 09:23 Thursday, January 21, 1999 1
Suffix=1 for Females; Suffix=2 for Males.

OBS	ID	GRP	AGE	TSH1	T31	T41	TSH2	T32	T42
1	3801	0.0	21D	0.54	118.93	4.57	0.90	120.00	5.66
2	3802	0.0	21D	0.56	127.92	2.80	.	.	.
3	3803	0.0	21D	1.64	93.98	4.37	0.85	99.80	4.97
4	3804	0.0	21D	0.87	112.45	3.55	1.14	104.22	3.83
5	3805	0.0	21D	1.16	114.92	5.82	0.97	112.25	4.76
6	3806	0.0	21D	0.74	95.62	4.24	1.02	98.19	4.09
7	3807	0.0	21D	1.30	107.53	4.34	0.97	104.56	4.46
8	3808	0.0	21D	1.53	100.83	4.66	1.34	109.78	5.27
9	3809	0.0	21D	1.07	107.58	4.42	1.90	86.13	3.07
10	3810	0.0	21D	0.86	102.97	4.53	1.03	99.47	5.02
11	3811	0.0	21D	0.91	122.60	4.05	1.03	110.48	4.54
12	3812	0.0	21D	1.19	104.24	3.55	1.61	99.33	4.41
13	3813	0.0	21D	1.74	103.20	3.18	1.66	116.19	3.59
14	3814	0.0	21D	0.85	109.83	3.14	1.59	118.50	5.95
15	3815	0.0	21D	0.69	88.40	2.48	0.56	103.55	2.67
16	3816	0.0	21D	2.74	85.37	3.32	2.30	96.28	3.17
17	3818	0.0	21D	0.85	104.36	4.89	1.17	116.60	4.52
18	3819	0.0	21D	1.16	101.32	3.59	0.98	94.88	3.10
19	3821	0.0	21D	0.84	79.83	4.37	0.58	111.49	3.81
20	3822	0.0	21D	0.57	90.65	3.09	0.70	97.51	3.49
21	3823	0.0	21D	1.18	107.12	3.08	1.16	105.90	2.55
22	3824	0.0	21D	1.45	117.65	4.68	1.93	108.85	5.97
23	3825	0.0	21D	0.73	104.83	5.96	1.17	115.59	4.89
24	3826	0.0	21D	1.51	105.71	5.29	1.59	85.59	3.77
25	3827	0.0	21D	1.35	115.38	5.59	1.84	114.60	5.76
26	3828	0.0	21D	0.16	137.69	6.65	1.12	125.29	5.41
27	3829	0.0	21D	1.70	115.59	4.80	1.59	106.54	5.60
28	3830	0.0	21D	1.48	90.20	4.54	0.71	97.65	4.56
29	3831	0.3	21D	0.84	118.75	5.65	1.16	140.77	5.55
30	3833	0.3	21D	1.47	122.11	6.87	1.02	102.38	3.45
31	3834	0.3	21D	0.74	105.27	4.72	0.50	98.34	3.77
32	3837	0.3	21D	1.61	109.33	4.62	1.12	109.50	4.00
33	3838	0.3	21D	0.76	116.85	3.92	0.48	104.64	3.91
34	3842	0.3	21D	0.96	116.94	4.97	0.83	116.33	4.59
35	3843	0.3	21D	0.91	123.21	5.54	1.00	126.47	4.72
36	3845	0.3	21D	0.47	95.72	4.43	0.63	77.78	3.68
37	3846	0.3	21D	1.62	99.48	4.52	0.97	100.00	4.52
38	3847	0.3	21D	1.14	139.51	4.28	.	.	.
39	3848	0.3	21D	1.11	99.10	4.77	0.97	83.62	3.07
40	3849	0.3	21D	1.33	125.37	5.77	0.89	117.49	5.07
41	3850	0.3	21D	1.84	85.45	4.11	0.72	91.03	4.41
42	3851	0.3	21D	1.16	105.31	3.56	0.98	116.70	5.70
43	3852	0.3	21D	1.27	106.74	4.42	0.58	109.25	3.97
44	3854	0.3	21D	1.27	116.06	5.47	0.91	128.06	6.23
45	3855	0.3	21D	0.89	119.82	4.15	0.66	135.82	4.82
46	3856	0.3	21D	1.00	114.33	3.58	1.04	119.85	3.95
47	3857	0.3	21D	1.52	93.59	4.84	1.48	115.96	6.37
48	3858	0.3	21D	1.73	104.69	6.25	2.00	117.18	5.60
49	3859	0.3	21D	1.30	111.68	6.70	1.05	126.16	6.00
50	3860	0.3	21D	1.20	88.98	3.89	0.77	96.81	3.53
51	3861	3.0	21D	1.19	94.02	5.40	0.38	96.85	4.73
52	3862	3.0	21D	1.28	100.02	3.30	0.88	91.83	3.51
53	3863	3.0	21D	1.67	104.22	5.34	1.07	115.12	5.69
54	3864	3.0	21D	0.70	81.32	3.63	0.95	86.32	3.81
55	3865	3.0	21D	1.15	88.17	4.35	1.20	86.70	4.04

Suffix=1 for Females; Suffix=2 for Males.

OBS	ID	GRP	AGE	TSH1	T31	T41	TSH2	T32	T42
56	3866	3	21D	1.21	104.71	4.07	0.81	114.95	4.22
57	3867	3	21D	0.61	111.35	4.11	0.67	101.21	3.34
58	3868	3	21D	1.43	109.34	3.28	0.93	85.71	5.11
59	3869	3	21D	0.98	113.15	4.25	0.99	100.07	4.33
60	3872	3	21D	1.48	117.39	5.01	1.34	117.67	4.26
61	3873	3	21D	2.15	118.55	4.18	1.27	114.37	4.86
62	3875	3	21D	1.40	102.32	4.54	0.99	135.10	5.59
63	3876	3	21D	1.62	142.56	4.44	1.38	126.16	4.60
64	3877	3	21D	1.23	125.24	3.94	0.70	127.60	4.77
65	3878	3	21D	0.70	112.91	4.06	0.63	139.04	4.65
66	3879	3	21D	0.90	109.09	3.78	0.60	114.07	3.87
67	3880	3	21D	0.67	89.02	4.96	0.56	101.60	5.67
68	3882	3	21D	0.82	116.22	6.23	0.83	123.71	5.71
69	3883	3	21D	1.01	120.88	3.90	0.80	119.86	4.95
70	3884	3	21D	1.30	131.74	3.78	1.17	120.28	4.03
71	3885	3	21D	0.62	98.85	4.46	0.72	96.82	4.53
72	3887	3	21D	0.86	110.08	4.12	0.88	126.66	4.59
73	3888	3	21D	1.00	108.36	5.20	0.69	102.72	4.37
74	3889	3	21D	1.36	108.76	5.06	0.76	90.42	5.54
75	3890	3	21D	1.18	114.05	2.70	0.72	110.42	2.56
76	3891	30	21D	1.11	101.34	3.64	1.10	103.06	4.09
77	3892	30	21D	1.47	106.42	2.84	.	.	.
78	3893	30	21D	1.01	96.20	4.49	0.74	118.93	5.40
79	3894	30	21D	1.50	110.06	3.96	1.55	107.78	4.94
80	3895	30	21D	2.05	89.95	4.46	1.68	94.67	5.01
81	3897	30	21D	1.32	94.62	3.17	1.20	101.91	4.31
82	3899	30	21D	1.29	94.64	4.82	0.95	91.54	4.67
83	3900	30	21D	1.34	95.71	2.83	1.22	86.49	2.85
84	3901	30	21D	0.60	82.98	2.91	0.74	79.34	4.10
85	3902	30	21D	1.11	95.47	3.52	1.04	94.80	3.59
86	3904	30	21D	1.11	90.80	2.94	1.01	93.58	4.68
87	3905	30	21D	.	.	.	1.71	117.53	2.64
88	3906	30	21D	1.14	87.52	3.42	0.89	100.49	4.94
89	3907	30	21D	1.54	79.28	2.67	1.63	127.13	6.03
90	3910	30	21D	2.14	124.86	4.21	2.43	127.13	6.70
91	3911	30	21D	1.19	90.74	2.88	1.02	104.62	3.62
92	3912	30	21D	1.19	102.83	3.78	1.44	104.71	2.74
93	3913	30	21D	1.70	98.04	4.67	1.62	95.74	5.66
94	3915	30	21D	1.65	106.41	4.65	1.24	118.72	5.75
95	3916	30	21D	1.10	115.86	4.31	1.18	101.66	3.69
96	3917	30	21D	0.93	97.81	5.24	1.28	115.02	5.51
97	3918	30	21D	1.01	93.00	4.54	1.36	142.11	5.34
98	3919	30	21D	1.34	80.96	6.74	0.98	104.32	4.04
99	3920	30	21D	1.09	108.86	3.31	1.20	138.88	3.78

1 MEANS of Generation F1, DAY 21 data for repeated measures. 3
Suffix=1 for Females; Suffix=2 for Males. 09:23 Thursday, January 21, 1999

GRP=0

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
TSH1	28	1.1203571	0.0964152	0.5101814	0.1600000	2.7400000
T31	28	105.9535714	2.4708986	13.0747665	79.8300000	137.6900000
T41	28	4.2696429	0.1926517	1.0194170	2.4800000	6.6500000
TSH2	27	1.2374074	0.0861880	0.4478461	0.5600000	2.3000000
T32	27	105.8970370	1.9197980	9.9755631	85.5900000	125.2900000
T42	27	4.4033333	0.1950272	1.0133911	2.5500000	5.9700000

GRP=0.3

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
TSH1	22	1.1881818	0.0750574	0.3520503	0.4700000	1.8400000
T31	22	109.9222727	2.7857661	13.0664011	85.4500000	139.5100000
T41	22	4.8650000	0.2016799	0.9459626	3.5600000	6.8700000
TSH2	21	0.9409524	0.0745396	0.3415831	0.4800000	2.0000000
T32	21	111.1495238	3.5726718	16.3720391	77.7800000	140.7700000
T42	21	4.6147619	0.2137150	0.9793652	3.0700000	6.3700000

GRP=3

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
TSH1	25	1.1408000	0.0749376	0.3746879	0.6100000	2.1500000
T31	25	109.2928000	2.7136494	13.5682468	81.3200000	142.5600000
T41	25	4.3236000	0.1555141	0.7775704	2.7000000	6.2300000
TSH2	25	0.8768000	0.0508747	0.2543737	0.3800000	1.3800000
T32	25	109.8104000	3.1385677	15.6928385	85.7100000	139.0400000
T42	25	4.5332000	0.1577939	0.7889694	2.5600000	5.7100000

GRP=30

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
TSH1	23	1.3013043	0.0732916	0.3514943	0.6000000	2.1400000
T31	23	97.5808696	2.3031636	11.0455844	79.2800000	124.8600000
T41	23	3.9130435	0.2049751	0.9830261	2.6700000	6.7400000
TSH2	23	1.2700000	0.0795342	0.3814327	0.7400000	2.4300000
T32	23	107.3982609	3.3487192	16.0598932	79.3400000	142.1100000
T42	23	4.5252174	0.2264661	1.0860934	2.6400000	6.7000000

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in data set = 99

NOTE: Observations with missing values will not be included in this analysis. Thus, only 95 observations can be used in this analysis.

General Linear Models Procedure

Dependent Variable: T41

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	9.51553209	3.17184403	3.66	0.0153
Error	91	78.81858370	0.86613828		
Corrected Total	94	88.33411579			

R-Square	C.V.	Root MSE	T41 Mean
0.107722	21.31723	0.93066551	4.36578947

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	9.51553209	3.17184403	3.66	0.0153

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	9.51553209	3.17184403	3.66	0.0153

General Linear Models Procedure

Dependent Variable: T42

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.72784090	0.24261363	0.27	0.8499
Error	91	83.05904963	0.91273681		
Corrected Total	94	83.78689053			

R-Square	C.V.	Root MSE	T42 Mean
0.008687	21.07913	0.95537260	4.53231579

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	0.72784090	0.24261363	0.27	0.8499

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	0.72784090	0.24261363	0.27	0.8499

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Repeated Measures Level Information

Dependent Variable	T41	T42
Level of SEX	1	2

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX Effect
H = Type III SS&CP Matrix for SEX E = Error SS&CP Matrix

S=1 M=-0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.97207577	2.6141	1	91	0.1094
Pillai's Trace	0.02792423	2.6141	1	91	0.1094
Hotelling-Lawley Trace	0.02872639	2.6141	1	91	0.1094
Roy's Greatest Root	0.02872639	2.6141	1	91	0.1094

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX*GRP Effect
H = Type III SS&CP Matrix for SEX*GRP E = Error SS&CP Matrix

S=1 M=0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.90340906	3.2432	3	91	0.0256
Pillai's Trace	0.09659094	3.2432	3	91	0.0256
Hotelling-Lawley Trace	0.10691828	3.2432	3	91	0.0256
Roy's Greatest Root	0.10691828	3.2432	3	91	0.0256

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Tests of Hypotheses for Between Subjects Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	5.48008956	1.82669652	1.42	0.2430
Error	91	117.32693992	1.28930703		

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F	Adjusted G - G	Pr > F H - F
SEX	1	1.27978048	1.27978048	2.61	0.1094	.	.
SEX*GRP	3	4.76328344	1.58776115	3.24	0.0256	.	.
Error(SEX)	91	44.55069341	0.48956806				

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Analysis of Variance of Contrast Variables

SEX.N represents the contrast between the nth level of SEX and the last

Contrast Variable: SEX.1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	2.55956097	2.55956097	2.61	0.1094
GRP	3	9.52656687	3.17552229	3.24	0.0256
Error	91	89.10138681	0.97913612		

1 Generation F1,DAY 21, T4 09:23 Thursday, January 21, 1999 11
Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T41

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 0.866138
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 0.7104
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	GRP
	A	4.8929	21	0.3
	A			
B	A	4.3241	27	0
B	A			
B	A	4.3236	25	3
B				
B		3.9618	22	30

1 Generation F1,DAY 21, T4 09:23 Thursday, January 21, 1999 12
Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T42

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 0.912737
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 0.7292
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	4.6148	21	0.3
A			
A	4.6109	22	30
A			
A	4.5332	25	3
A			
A	4.4033	27	0

1 Generation F1,DAY 21, T3 09:23 Thursday, January 21, 1999 13
Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in data set = 99

NOTE: Observations with missing values will not be included in this analysis. Thus, only 95 observations can be used in this analysis.

1 Generation F1,DAY 21, T3 09:23 Thursday, January 21, 1999 14
Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Dependent Variable: T31

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2064.38182225	688.12727408	4.54	0.0052
Error	91	13804.23115248	151.69484783		
Corrected Total	94	15868.61297474			

R-Square	C.V.	Root MSE	T31 Mean
0.130092	11.71489	12.31644623	105.13494737

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	2064.38182225	688.12727408	4.54	0.0052

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	2064.38182225	688.12727408	4.54	0.0052

1 Generation F1,DAY 21, T3 09:23 Thursday, January 21, 1999 15
Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Dependent Variable: T32

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	424.20015417	141.40005139	0.66	0.5773
Error	91	19425.47154057	213.46672023		
Corrected Total	94	19849.67169474			

R-Square	C.V.	Root MSE	T32 Mean
0.021371	13.48716	14.61050034	108.32894737

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	424.20015417	141.40005139	0.66	0.5773

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	424.20015417	141.40005139	0.66	0.5773

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Repeated Measures Level Information

Dependent Variable	T31	T32
Level of SEX	1	2

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX Effect
H = Type III SS&CP Matrix for SEX E = Error SS&CP Matrix

S=1 M=-0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.93817904	5.9964	1	91	0.0163
Pillai's Trace	0.06182096	5.9964	1	91	0.0163
Hotelling-Lawley Trace	0.06589462	5.9964	1	91	0.0163
Roy's Greatest Root	0.06589462	5.9964	1	91	0.0163

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX*GRP Effect
H = Type III SS&CP Matrix for SEX*GRP E = Error SS&CP Matrix

S=1 M=0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.92796295	2.3548	3	91	0.0772
Pillai's Trace	0.07203705	2.3548	3	91	0.0772
Hotelling-Lawley Trace	0.07762923	2.3548	3	91	0.0772
Roy's Greatest Root	0.07762923	2.3548	3	91	0.0772

Generation F1,DAY 21, T3
Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Tests of Hypotheses for Between Subjects Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	1841.56332307	613.85444102	2.24	0.0885
Error	91	24894.97280640	273.57112974		

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F	Adjusted G - G	Pr > F H - F
SEX	1	549.21389263	549.21389263	6.00	0.0163	.	.
SEX*GRP	3	647.01865335	215.67288445	2.35	0.0772	.	.
Error(SEX)	91	8334.72988665	91.59043831				

General Linear Models Procedure
Repeated Measures Analysis of Variance
Analysis of Variance of Contrast Variables

SEX.N represents the contrast between the nth level of SEX and the last

Contrast Variable: SEX.1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	1098.42778526	1098.42778526	6.00	0.0163
GRP	3	1294.03730671	431.34576890	2.35	0.0772
Error	91	16669.45977329	183.18087663		

1 Generation F1,DAY 21, T3 09:23 Thursday, January 21, 1999 20
Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T31

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 151.6948
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 9.4008
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	109.293	25	3
A			
A	108.513	21	0.3
A			
B A	105.140	27	0
B			
B	97.179	22	30

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T32

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 213.4667
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 11.152
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	111.150	21	0.3
A			
A	109.810	25	3
A			
A	106.938	22	30
A			
A	105.897	27	0

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in data set = 99

NOTE: Observations with missing values will not be included in this analysis. Thus, only 95 observations can be used in this analysis.

General Linear Models Procedure

Dependent Variable: TSH1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.36063343	0.12021114	0.71	0.5472
Error	91	15.36005500	0.16879181		
Corrected Total	94	15.72068842			

R-Square	C.V.	Root MSE	TSH1 Mean
0.022940	34.60419	0.41084281	1.18726316

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	0.36063343	0.12021114	0.71	0.5472

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	0.36063343	0.12021114	0.71	0.5472

1 Generation F1,DAY 21, TSH 09:23 Thursday, January 21, 1999 24
Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Dependent Variable: TSH2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2.74305548	0.91435183	6.88	0.0003
Error	91	12.09964347	0.13296312		
Corrected Total	94	14.84269895			

R-Square	C.V.	Root MSE	TSH2 Mean
0.184808	33.76635	0.36464108	1.07989474

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	2.74305548	0.91435183	6.88	0.0003

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	2.74305548	0.91435183	6.88	0.0003

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Repeated Measures Level Information

Dependent Variable	TSH1	TSH2
Level of SEX	1	2

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX Effect
H = Type III SS&CP Matrix for SEX E = Error SS&CP Matrix

S=1 M=-0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.89351379	10.8451	1	91	0.0014
Pillai's Trace	0.10648621	10.8451	1	91	0.0014
Hotelling-Lawley Trace	0.11917691	10.8451	1	91	0.0014
Roy's Greatest Root	0.11917691	10.8451	1	91	0.0014

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX*GRP Effect
H = Type III SS&CP Matrix for SEX*GRP E = Error SS&CP Matrix

S=1 M=0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.82339898	6.5058	3	91	0.0005
Pillai's Trace	0.17660102	6.5058	3	91	0.0005
Hotelling-Lawley Trace	0.21447806	6.5058	3	91	0.0005
Roy's Greatest Root	0.21447806	6.5058	3	91	0.0005

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Tests of Hypotheses for Between Subjects Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	1.98018483	0.66006161	2.70	0.0501
Error	91	22.22138149	0.24419101		

1

Generation F1,DAY 21, TSH
Suffix=1 for Females; Suffix=2 for Males.

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General Linear Models Procedure
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F	Adjusted G - G	Pr > F H - F
SEX	1	0.62428642	0.62428642	10.85	0.0014	.	.
SEX*GRP	3	1.12350407	0.37450136	6.51	0.0005	.	.
Error(SEX)	91	5.23831698	0.05756392				

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Analysis of Variance of Contrast Variables

SEX.N represents the contrast between the nth level of SEX and the last

Contrast Variable: SEX.1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	1.24857284	1.24857284	10.85	0.0014
GRP	3	2.24700815	0.74900272	6.51	0.0005
Error	91	10.47663396	0.11512785		

1

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Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH1

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 0.168792
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 0.3136
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	1.2936	22	30
A			
A	1.1905	21	0.3
A			
A	1.1411	27	0
A			
A	1.1408	25	3

1 Generation F1,DAY 21, TSH 09:23 Thursday, January 21, 1999 30
Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH2

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 0.132963
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 0.2783
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	1.2500	22	30
A			
A	1.2374	27	0
B	0.9410	21	0.3
B			
B	0.8768	25	3

1 DATA FROM F0 GENERATION 09:23 Thursday, January 21, 1999 31

OBS	ID	GRP	SEX	AGE	TSH	T3	T4
1	3801	0	F	5M	0.72	27.62	1.79
2	3802	0	F	5M	3.51	88.20	4.35
3	3803	0	F	5M	0.84	23.88	1.05
4	3804	0	F	5M	1.66	55.70	1.45
5	3805	0	F	5M	1.31	56.03	2.47
6	3806	0	F	5M	2.46	36.57	2.23
7	3807	0	F	5M	1.82	48.65	2.04
8	3808	0	F	5M	1.59	62.75	2.98
9	3809	0	F	5M	1.39	62.30	2.02
10	3810	0	F	5M	2.41	44.70	2.80
11	3811	0	F	5M	1.44	50.48	2.06
12	3812	0	F	5M	1.86	47.12	1.68
13	3813	0	F	5M	2.77	43.52	2.43
14	3814	0	F	5M	1.36	52.48	2.16
15	3815	0	F	5M	1.80	68.54	2.10
16	3816	0	F	5M	3.16	108.80	1.58
17	3817	0	F	5M	1.31	68.48	2.64
18	3818	0	F	5M	1.50	159.25	1.45
19	3819	0	F	5M	2.59	41.89	1.45
20	3820	0	F	5M	1.59	114.68	3.17
21	3821	0	F	5M	2.08	44.07	1.11
22	3822	0	F	5M	5.24	60.97	1.93
23	3823	0	F	5M	1.97	43.95	1.90
24	3824	0	F	5M	1.83	24.39	1.34
25	3825	0	F	5M	2.05	53.42	1.88
26	3826	0	F	5M	2.41	40.63	2.33
27	3827	0	F	5M	2.00	38.53	1.83
28	3828	0	F	5M	2.20	47.03	2.46
29	3829	0	F	5M	2.82	60.18	2.44
30	3830	0	F	5M	1.93	58.28	2.67
31	3601	0	M	6M	1.19	88.35	5.07
32	3602	0	M	6M	0.93	84.17	4.35
33	3603	0	M	6M	0.62	79.49	5.66
34	3604	0	M	6M	0.80	82.88	5.33
35	3605	0	M	6M	2.16	86.13	5.19
36	3606	0	M	6M	1.18	93.42	4.36
37	3607	0	M	6M	1.05	63.72	3.81
38	3608	0	M	6M	1.80	69.21	4.18
39	3609	0	M	6M	4.41	72.29	4.12
40	3610	0	M	6M	1.40	68.40	4.82
41	3611	0	M	6M	0.84	61.11	4.24
42	3612	0	M	6M	1.45	61.69	4.84
43	3613	0	M	6M	0.56	71.49	4.94
44	3614	0	M	6M	1.81	83.01	4.56
45	3615	0	M	6M	1.08	77.97	5.39
46	3616	0	M	6M	3.04	69.67	3.84
47	3617	0	M	6M	1.34	71.30	4.95
48	3618	0	M	6M	2.08	51.76	3.18
49	3619	0	M	6M	0.42	68.45	4.49
50	3620	0	M	6M	0.95	66.34	5.01
51	3621	0	M	6M	2.42	62.40	4.30
52	3622	0	M	6M	1.48	96.45	4.44
53	3623	0	M	6M	3.67	61.89	4.17
54	3624	0	M	6M	0.35	73.45	4.35
55	3625	0	M	6M	1.88	80.55	4.11
56	3627	0	M	6M	1.35	55.93	5.37

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OBS	ID	GRP	SEX	AGE	TSH	T3	T4
57	3628	0.0	M	6M	0.90	65.47	5.25
58	3629	0.0	M	6M	0.75	59.35	4.87
59	3630	0.0	M	6M	2.46	77.51	5.40
60	3831	0.3	F	5M	1.46	46.98	1.38
61	3832	0.3	F	5M	2.99	76.19	3.82
62	3833	0.3	F	5M	1.42	118.14	2.13
63	3834	0.3	F	5M	1.40	39.12	3.15
64	3835	0.3	F	5M	1.64	78.71	4.42
65	3836	0.3	F	5M	1.48	102.72	3.65
66	3837	0.3	F	5M	1.30	50.23	3.71
67	3838	0.3	F	5M	2.02	54.58	2.16
68	3839	0.3	F	5M	1.16	82.60	4.07
69	3840	0.3	F	5M	2.40	132.21	5.87
70	3841	0.3	F	5M	1.71	67.83	3.55
71	3842	0.3	F	5M	2.67	50.68	2.93
72	3843	0.3	F	5M	4.82	42.96	2.44
73	3844	0.3	F	5M	1.88	141.56	3.76
74	3845	0.3	F	5M	1.07	43.53	1.14
75	3846	0.3	F	5M	1.26	49.05	2.57
76	3847	0.3	F	5M	1.63	84.55	2.96
77	3848	0.3	F	5M	2.78	54.17	2.11
78	3849	0.3	F	5M	1.13	43.88	2.48
79	3850	0.3	F	5M	2.72	46.08	2.92
80	3851	0.3	F	5M	3.71	45.33	1.66
81	3852	0.3	F	5M	1.76	34.24	1.54
82	3853	0.3	F	5M	2.51	101.45	3.29
83	3854	0.3	F	5M	1.34	42.79	2.46
84	3855	0.3	F	5M	1.62	28.25	1.42
85	3856	0.3	F	5M	3.47	40.06	3.48
86	3857	0.3	F	5M	2.79	46.68	3.64
87	3858	0.3	F	5M	4.32	69.99	2.02
88	3859	0.3	F	5M	3.17	58.12	2.98
89	3860	0.3	F	5M	2.77	70.99	3.39
90	3631	0.3	M	6M	1.83	79.54	4.79
91	3632	0.3	M	6M	0.91	77.36	4.51
92	3633	0.3	M	6M	1.30	131.87	7.01
93	3634	0.3	M	6M	0.81	79.14	4.28
94	3635	0.3	M	6M	2.09	110.78	6.17
95	3636	0.3	M	6M	1.02	79.79	4.55
96	3637	0.3	M	6M	1.69	98.71	4.28
97	3638	0.3	M	6M	0.82	107.52	4.74
98	3639	0.3	M	6M	2.38	82.07	4.28
99	3640	0.3	M	6M	2.14	80.26	3.94
100	3641	0.3	M	6M	1.82	57.22	3.30
101	3642	0.3	M	6M	1.79	87.48	3.75
102	3643	0.3	M	6M	0.65	120.54	4.83
103	3644	0.3	M	6M	1.67	83.31	4.74
104	3645	0.3	M	6M	2.64	91.05	5.01
105	3646	0.3	M	6M	1.08	102.18	5.81
106	3647	0.3	M	6M	1.42	79.21	4.57
107	3648	0.3	M	6M	1.80	72.73	4.18
108	3649	0.3	M	6M	1.26	68.76	4.86
109	3650	0.3	M	6M	0.71	89.35	5.05
110	3651	0.3	M	6M	0.72	78.85	4.25
111	3652	0.3	M	6M	0.61	92.34	5.56
112	3653	0.3	M	6M	2.29	91.63	5.07

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OBS	ID	GRP	SEX	AGE	TSH	T3	T4
113	3654	0.3	M	6M	0.31	109.01	5.63
114	3655	0.3	M	6M	0.78	80.77	4.05
115	3656	0.3	M	6M	1.08	70.67	3.80
116	3657	0.3	M	6M	1.43	91.65	5.36
117	3658	0.3	M	6M	1.17	73.19	4.18
118	3659	0.3	M	6M	0.29	74.18	3.57
119	3660	0.3	M	6M	2.07	80.52	5.66
120	3861	3.0	F	5M	1.71	47.40	3.05
121	3862	3.0	F	5M	3.25	65.02	3.67
122	3863	3.0	F	5M	0.87	61.89	2.24
123	3864	3.0	F	5M	0.62	47.74	1.36
124	3865	3.0	F	5M	2.36	42.31	2.07
125	3866	3.0	F	5M	1.46	49.32	2.56
126	3867	3.0	F	5M	2.72	49.26	2.15
127	3868	3.0	F	5M	2.26	48.82	2.20
128	3869	3.0	F	5M	1.09	45.46	2.24
129	3870	3.0	F	5M	1.80	89.35	3.60
130	3871	3.0	F	5M	1.52	85.38	4.03
131	3872	3.0	F	5M	2.11	58.63	2.69
132	3873	3.0	F	5M	2.29	45.69	2.68
133	3874	3.0	F	5M	1.92	90.35	3.97
134	3875	3.0	F	5M	2.16	53.03	3.06
135	3876	3.0	F	5M	2.88	42.69	1.46
136	3877	3.0	F	5M	3.26	52.51	2.74
137	3878	3.0	F	5M	1.50	63.21	4.25
138	3879	3.0	F	5M	1.27	38.87	1.64
139	3880	3.0	F	5M	1.44	53.24	2.68
140	3882	3.0	F	5M	1.41	58.15	2.82
141	3883	3.0	F	5M	3.87	46.05	2.90
142	3884	3.0	F	5M	1.19	75.50	3.59
143	3885	3.0	F	5M	1.44	43.87	3.15
144	3886	3.0	F	5M	2.53	70.42	4.63
145	3887	3.0	F	5M	2.46	47.75	3.83
146	3888	3.0	F	5M	1.38	50.40	2.42
147	3889	3.0	F	5M	2.27	59.71	3.69
148	3890	3.0	F	5M	2.67	52.14	3.43
149	3661	3.0	M	6M	1.29	81.05	5.05
150	3662	3.0	M	6M	2.84	99.22	3.73
151	3663	3.0	M	6M	1.54	92.30	6.09
152	3664	3.0	M	6M	1.36	102.47	4.29
153	3665	3.0	M	6M	1.71	102.42	5.34
154	3666	3.0	M	6M	0.68	67.87	4.79
155	3667	3.0	M	6M	1.39	88.38	4.39
156	3668	3.0	M	6M	0.39	68.59	4.96
157	3669	3.0	M	6M	1.22	75.98	5.99
158	3670	3.0	M	6M	1.91	98.88	4.76
159	3671	3.0	M	6M	3.76	82.85	4.58
160	3672	3.0	M	6M	0.87	90.33	5.27
161	3673	3.0	M	6M	1.40	90.58	4.17
162	3674	3.0	M	6M	1.32	90.37	4.38
163	3675	3.0	M	6M	1.03	84.18	4.57
164	3676	3.0	M	6M	0.90	72.26	4.74
165	3677	3.0	M	6M	0.87	79.72	4.73
166	3678	3.0	M	6M	0.38	69.36	3.34
167	3679	3.0	M	6M	2.47	85.77	3.79
168	3680	3.0	M	6M	1.36	74.30	5.45

1 DATA FROM F0 GENERATION 09:23 Thursday, January 21, 1999 34

OBS	ID	GRP	SEX	AGE	TSH	T3	T4
169	3681	3	M	6M	2.58	92.89	4.40
170	3682	3	M	6M	2.19	98.91	6.02
171	3683	3	M	6M	2.07	111.73	4.99
172	3684	3	M	6M	0.77	70.66	4.27
173	3685	3	M	6M	0.45	166.80	3.47
174	3686	3	M	6M	3.03	81.70	5.52
175	3687	3	M	6M	0.96	81.88	4.39
176	3688	3	M	6M	1.47	94.46	6.49
177	3689	3	M	6M	1.05	79.94	4.74
178	3690	3	M	6M	1.35	77.72	3.62
179	3891	30	F	5M	2.33	37.30	1.90
180	3892	30	F	5M	2.04	56.00	3.92
181	3893	30	F	5M	1.27	45.02	3.02
182	3894	30	F	5M	2.99	43.84	1.83
183	3895	30	F	5M	2.59	40.47	3.06
184	3896	30	F	5M	1.34	68.29	3.24
185	3897	30	F	5M	1.20	55.97	2.39
186	3898	30	F	5M	2.67	108.40	3.11
187	3899	30	F	5M	1.52	60.10	3.29
188	3900	30	F	5M	1.51	42.03	2.28
189	3901	30	F	5M	1.44	38.32	1.83
190	3902	30	F	5M	1.27	46.37	2.25
191	3903	30	F	5M	3.33	89.67	2.39
192	3904	30	F	5M	1.66	54.31	2.59
193	3905	30	F	5M	2.47	91.70	4.62
194	3906	30	F	5M	3.02	47.31	2.46
195	3907	30	F	5M	2.64	64.16	1.88
196	3908	30	F	5M	2.14	107.67	2.78
197	3909	30	F	5M	1.80	100.45	2.54
198	3910	30	F	5M	3.30	49.36	1.58
199	3911	30	F	5M	1.53	79.20	2.98
200	3912	30	F	5M	1.80	88.74	1.59
201	3913	30	F	5M	3.06	41.83	1.57
202	3914	30	F	5M	1.51	77.67	2.47
203	3915	30	F	5M	3.58	50.85	1.08
204	3916	30	F	5M	1.45	47.37	1.53
205	3917	30	F	5M	1.82	47.81	2.06
206	3918	30	F	5M	2.49	42.50	2.93
207	3919	30	F	5M	1.98	39.32	2.28
208	3920	30	F	5M	3.46	49.17	1.19
209	3691	30	M	6M	2.58	64.35	3.66
210	3692	30	M	6M	3.24	89.66	3.66
211	3693	30	M	6M	2.70	91.40	3.10
212	3694	30	M	6M	5.17	95.93	3.94
213	3695	30	M	6M	3.36	85.61	3.95
214	3696	30	M	6M	1.28	83.01	4.05
215	3697	30	M	6M	8.21	100.77	6.08
216	3699	30	M	6M	2.07	60.94	3.23
217	3700	30	M	6M	1.94	59.68	2.63
218	3701	30	M	6M	1.76	81.16	3.52
219	3702	30	M	6M	3.82	92.08	3.39
220	3703	30	M	6M	3.74	98.18	3.63
221	3704	30	M	6M	1.76	80.75	4.37
222	3705	30	M	6M	2.29	68.34	2.62
223	3706	30	M	6M	1.97	74.23	3.00
224	3707	30	M	6M	5.37	61.49	3.27

1 DATA FROM F0 GENERATION 09:23 Thursday, January 21, 1999 35

OBS	ID	GRP	SEX	AGE	TSH	T3	T4
225	3708	30	M	6M	1.13	84.65	2.10
226	3709	30	M	6M	14.15	71.72	1.57
227	3710	30	M	6M	13.31	74.04	2.99
228	3711	30	M	6M	1.14	71.23	4.50
229	3712	30	M	6M	1.93	70.55	4.18
230	3713	30	M	6M	7.40	71.35	4.45
231	3714	30	M	6M	1.05	64.68	3.00
232	3715	30	M	6M	1.79	116.75	4.08
233	3716	30	M	6M	1.97	89.85	4.68
234	3717	30	M	6M	0.94	66.63	3.19
235	3718	30	M	6M	8.27	78.42	3.95
236	3719	30	M	6M	6.87	54.88	3.35
237	3720	30	M	6M	1.04	76.21	3.61

1 F0 Generation, Means by SEX 09:23 Thursday, January 21, 1999 36

SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	119	3860.33	3.1975080	34.8806948	3801.00	3920.00
GRP	119	8.3697479	1.1609977	12.6649895	0	30.0000000
TSH	119	2.1087395	0.0773778	0.8440927	0.6200000	5.2400000
T3	119	59.8497479	2.2107705	24.1166592	23.8800000	159.2500000
T4	119	2.5910084	0.0827259	0.9024331	1.0500000	5.8700000

SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	118	3660.47	3.2002262	34.7633543	3601.00	3720.00
GRP	118	8.2118644	1.1549327	12.5457806	0	30.0000000
TSH	118	2.0492373	0.1953372	2.1219049	0.2900000	14.1500000
T3	118	81.8444068	1.5246098	16.5615013	51.7600000	166.8000000
T4	118	4.4274576	0.0832387	0.9042040	1.5700000	7.0100000

GRP=0

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	59	3717.02	13.2011036	101.3996007	3601.00	3830.00
TSH	59	1.7964407	0.1230441	0.9451195	0.3500000	5.2400000
T3	59	65.0328814	2.9519008	22.6739804	23.8800000	159.2500000
T4	59	3.3623729	0.1841824	1.4147320	1.0500000	5.6600000

GRP=0.3

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	60	3745.50	13.0675667	101.2209364	3631.00	3860.00
TSH	60	1.7830000	0.1202247	0.9312563	0.2900000	4.8200000
T3	60	76.0891667	3.3691531	26.0973476	28.2500000	141.5600000
T4	60	3.8146667	0.1684937	1.3051469	1.1400000	7.0100000

GRP=3

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	59	3773.71	13.1659384	101.1294921	3661.00	3890.00
TSH	59	1.7342373	0.1077443	0.8275998	0.3800000	3.8700000
T3	59	72.6733898	2.9975333	23.0244899	38.8700000	166.8000000
T4	59	3.8494915	0.1592210	1.2230000	1.3600000	6.4900000

GRP=30

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	59	3807.32	13.1611298	101.0925559	3691.00	3920.00
TSH	59	3.0077966	0.3421046	2.6277551	0.9400000	14.1500000
T3	59	69.3176271	2.6843263	20.6187013	37.3000000	116.7500000
T4	59	2.9896610	0.1308737	1.0052602	1.0800000	6.0800000

GRP=0 SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3815.50	1.6072751	8.8034084	3801.00	3830.00
TSH	30	2.0540000	0.1594023	0.8730825	0.7200000	5.2400000
T3	30	57.7696667	5.1493264	28.2040220	23.8800000	159.2500000
T4	30	2.1263333	0.1236620	0.6773248	1.0500000	4.3500000

GRP=0 SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	29	3615.14	1.6209255	8.7289508	3601.00	3630.00
TSH	29	1.5300000	0.1777452	0.9571871	0.3500000	4.4100000
T3	29	72.5465517	2.0850083	11.2281134	51.7600000	96.4500000
T4	29	4.6410345	0.1083501	0.5834830	3.1800000	5.6600000

GRP=0.3 SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3845.50	1.6072751	8.8034084	3831.00	3860.00
TSH	30	2.2133333	0.1801745	0.9868561	1.0700000	4.8200000
T3	30	64.7890000	5.3452191	29.2769705	28.2500000	141.5600000
T4	30	2.9033333	0.1896520	1.0387670	1.1400000	5.8700000

GRP=0.3 SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3645.50	1.6072751	8.8034084	3631.00	3660.00
TSH	30	1.3526667	0.1165263	0.6382408	0.2900000	2.6400000
T3	30	87.3893333	2.9681270	16.2571009	57.2200000	131.8700000
T4	30	4.7260000	0.1492407	0.8174249	3.3000000	7.0100000

GRP=3 SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	29	3875.31	1.6520650	8.8966424	3861.00	3890.00
TSH	29	1.9900000	0.1435424	0.7729997	0.6200000	3.8700000
T3	29	56.3503448	2.5982097	13.9917875	38.8700000	90.3500000
T4	29	2.9241379	0.1562495	0.8414296	1.3600000	4.6300000

GRP=3 SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3675.50	1.6072751	8.8034084	3661.00	3690.00
TSH	30	1.4870000	0.1488030	0.8150276	0.3800000	3.7600000
T3	30	88.4523333	3.4021201	18.6341793	67.8700000	166.8000000
T4	30	4.7440000	0.1442050	0.7898433	3.3400000	6.4900000

GRP=30 SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3905.50	1.6072751	8.8034084	3891.00	3920.00
TSH	30	2.1736667	0.1357876	0.7437393	1.2000000	3.5800000
T3	30	60.3733333	4.0112100	21.9703018	37.3000000	108.4000000
T4	30	2.4213333	0.1445999	0.7920063	1.0800000	4.6200000

GRP=30 SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	29	3705.76	1.6420094	8.8424915	3691.00	3720.00
TSH	29	3.8706897	0.6489744	3.4948339	0.9400000	14.1500000
T3	29	78.5703448	2.6672331	14.3634900	54.8800000	116.7500000
T4	29	3.5775862	0.1596908	0.8599612	1.5700000	6.0800000

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3
SEX	2	F M

Number of observations in data set = 237

General Linear Models Procedure

Dependent Variable: T4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	241.25464107	34.46494872	52.50	0.0001
Error	229	150.32005345	0.65641945		
Corrected Total	236	391.57469451			

R-Square	C.V.	Root MSE	T4 Mean
0.616114	23.11310	0.81019717	3.50535865

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	29.62445542	9.87481847	15.04	0.0001
SEX	1	198.02136032	198.02136032	301.67	0.0001
GRP*SEX	3	13.60882533	4.53627511	6.91	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	28.07875642	9.35958547	14.26	0.0001
SEX	1	198.01581342	198.01581342	301.66	0.0001
GRP*SEX	3	13.60882533	4.53627511	6.91	0.0002

General Linear Models Procedure

Dependent Variable: T3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	34937.85388704	4991.12198386	12.10	0.0001
Error	229	94446.22560494	412.42893277		
Corrected Total	236	129384.07949198			

R-Square	C.V.	Root MSE	T3 Mean
0.270032	28.68383	20.30834638	70.80067511

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	3977.55083365	1325.85027788	3.21	0.0237
SEX	1	28467.84975054	28467.84975054	69.02	0.0001
GRP*SEX	3	2492.45330286	830.81776762	2.01	0.1127

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	3811.29675945	1270.43225315	3.08	0.0283
SEX	1	28458.69687677	28458.69687677	69.00	0.0001
GRP*SEX	3	2492.45330286	830.81776762	2.01	0.1127

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General Linear Models Procedure

Dependent Variable: TSH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	129.23442772	18.46206110	8.77	0.0001
Error	229	481.83968621	2.10410343		
Corrected Total	236	611.07411392			

R-Square	C.V.	Root MSE	TSH Mean
0.211487	69.76784	1.45055280	2.07911392

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	67.87744714	22.62581571	10.75	0.0001
SEX	1	0.15498515	0.15498515	0.07	0.7863
GRP*SEX	3	61.20199542	20.40066514	9.70	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	69.42116553	23.14038851	11.00	0.0001
SEX	1	0.13455387	0.13455387	0.06	0.8006
GRP*SEX	3	61.20199542	20.40066514	9.70	0.0001

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T4

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 0.656419
Critical Value of Studentized Range= 3.660
Minimum Significant Difference= 0.3852
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 59.24686

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	3.8495	59	3
A			
A	3.8147	60	0.3
B	3.3624	59	0
B			
B	2.9897	59	30

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General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T3

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 412.4289
Critical Value of Studentized Range= 3.660
Minimum Significant Difference= 9.6566
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 59.24686

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	GRP
	A	76.089	60	0.3
	A			
B	A	72.673	59	3
B	A			
B	A	69.318	59	30
B				
B		65.033	59	0

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 2.104103
Critical Value of Studentized Range= 3.660
Minimum Significant Difference= 0.6897
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 59.24686

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	3.0078	59	30
B	1.7964	59	0
B			
B	1.7830	60	0.3
B			
B	1.7342	59	3

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T4

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 0.656419
Critical Value of Studentized Range= 2.787
Minimum Significant Difference= 0.2074
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 118.4979

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	SEX
A	4.4275	118	M
B	2.5910	119	F

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T3

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 412.4289
Critical Value of Studentized Range= 2.787
Minimum Significant Difference= 5.1986
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 118.4979

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	SEX
A	81.844	118	M
B	59.850	119	F

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 2.104103
Critical Value of Studentized Range= 2.787
Minimum Significant Difference= 0.3713
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 118.4979

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	SEX
A	2.1087	119	F
A			
A	2.0492	118	M

Level of GRP	Level of SEX	N	T4 Mean	T4 SD	T3 Mean	T3 SD	TSH Mean	TSH SD
0	F	30	2.12633333	0.67732477	57.7696667	28.2040220	2.05400000	0.87308253
0	M	29	4.64103448	0.58348304	72.5465517	11.2281134	1.53000000	0.95718710
3	F	29	2.92413793	0.84142955	56.3503448	13.9917875	1.99000000	0.77299972
3	M	30	4.74400000	0.78984328	88.4523333	18.6341793	1.48700000	0.81502761
30	F	30	2.42133333	0.79200633	60.3733333	21.9703018	2.17366667	0.74373931
30	M	29	3.57758621	0.85996119	78.5703448	14.3634900	3.87068966	3.49483387
0.3	F	30	2.90333333	1.03876695	64.7890000	29.2769705	2.21333333	0.98685615
0.3	M	30	4.72600000	0.81742489	87.3893333	16.2571009	1.35266667	0.63824076

---------- SEX=F ----------

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in by group = 119

SEX=F

General Linear Models Procedure

Dependent Variable: T4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	13.48606554	4.49535518	6.26	0.0006
Error	115	82.61141345	0.71836012		
Corrected Total	118	96.09747899			

R-Square	C.V.	Root MSE	T4 Mean
0.140337	32.71164	0.84756128	2.59100840

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	13.48606554	4.49535518	6.26	0.0006

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	13.48606554	4.49535518	6.26	0.0006

SEX=F

General Linear Models Procedure

Dependent Variable: T3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1225.04156255	408.34718752	0.70	0.5559
Error	115	67405.32192989	586.13323417		
Corrected Total	118	68630.36349244			

R-Square	C.V.	Root MSE	T3 Mean
0.017850	40.45161	24.21018864	59.84974790

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	1225.04156255	408.34718752	0.70	0.5559

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	1225.04156255	408.34718752	0.70	0.5559

---------- SEX=F ----------

General Linear Models Procedure

Dependent Variable: TSH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.95342759	0.31780920	0.44	0.7250
Error	115	83.12068333	0.72278855		
Corrected Total	118	84.07411092			

R-Square	C.V.	Root MSE	TSH Mean
0.011340	40.31649	0.85016972	2.10873950

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	0.95342759	0.31780920	0.44	0.7250

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	0.95342759	0.31780920	0.44	0.7250

---------- SEX=F ----------

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T4

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 115 MSE= 0.71836
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 0.573
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.74359

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	2.9241	29	3
A			
A	2.9033	30	0.3
A			
B A	2.4213	30	30
B			
B	2.1263	30	0

---------- SEX=F ----------

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T3

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 115 MSE= 586.1332
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 16.367
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.74359

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	64.789	30	0.3
A			
A	60.373	30	30
A			
A	57.770	30	0
A			
A	56.350	29	3

---------- SEX=F ----------

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 115 MSE= 0.722789
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 0.5747
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.74359

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	2.2133	30	0.3
A			
A	2.1737	30	30
A			
A	2.0540	30	0
A			
A	1.9900	29	3

---------- SEX=M ----------

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in by group = 118

Analysis by Sex

---------- SEX=M ----------

General Linear Models Procedure

Dependent Variable: T4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	27.94879729	9.31626576	15.69	0.0001
Error	114	67.70864000	0.59393544		
Corrected Total	117	95.65743729			

R-Square	C.V.	Root MSE	T4 Mean
0.292176	17.40665	0.77067207	4.42745763

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	27.94879729	9.31626576	15.69	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	27.94879729	9.31626576	15.69	0.0001

SEX=M

General Linear Models Procedure

Dependent Variable: T3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	5050.24523342	1683.41507781	7.10	0.0002
Error	114	27040.90367506	237.20090943		
Corrected Total	117	32091.14890847			

R-Square	C.V.	Root MSE	T3 Mean
0.157372	18.81781	15.40132817	81.84440678

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	5050.24523342	1683.41507781	7.10	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	5050.24523342	1683.41507781	7.10	0.0002

SEX=M

General Linear Models Procedure

Dependent Variable: TSH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	128.07122848	42.69040949	12.21	0.0001
Error	114	398.71900287	3.49753511		
Corrected Total	117	526.79023136			

R-Square	C.V.	Root MSE	TSH Mean
0.243116	91.26175	1.87016981	2.04923729

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	128.07122848	42.69040949	12.21	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	128.07122848	42.69040949	12.21	0.0001

---------- SEX=M ----------

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T4

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 114 MSE= 0.593935
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 0.5233
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.49153

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	4.7440	30	3
A			
A	4.7260	30	0.3
A			
A	4.6410	29	0
B	3.5776	29	30

---------- SEX=M ----------

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T3

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 114 MSE= 237.2009
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 10.457
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.49153

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	88.452	30	3
A			
A	87.389	30	0.3
A			
B A	78.570	29	30
B			
B	72.547	29	0

---------- SEX=M ----------

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 114 MSE= 3.497535
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 1.2698
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.49153

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	3.8707	29	30
B	1.5300	29	0
B			
B	1.4870	30	3
B			
B	1.3527	30	0.3

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

DATE: February 1, 1999

SUBJECT: Statistical analysis of ammonium perchlocate experiment

FROM: Dennis E. House Dennis E. House
NHEERL/BRSS/MD-55

TO: Andrew Geller
NHEERL/NTD/MD-74B

"Attached is the statistical analysis of the hormone data from the Argus Rat Developmental Neurotoxicology Study (Argus, 1998b). A memo from Argus Laboratories (RE: Argus Protocol #1416-001, 20 November 1998) contains thyroid hormone and thyrotrophin data from the Oral (Drinking Water) Two-Generation reproduction Study of ammonium perchlorate in the rat. Data were supplied on diskette in the form of ASCII text reports, one report for each gender/age group, and imported in ASCII form to SAS for further analysis.

The following is a statistical analysis of the thyroid and pituitary hormone data (T4, thyroxine; T3, triiodothyronine; TSH, thyroid stimulating hormone) found in that report. At the time of this analysis, data were available from both the F0 generation, females and males sacrificed at 5 and 6 months of age, respectively, and the F1 generation, one male and one female from each litter, sacrificed on postnatal day 21 (PND21). Males were sacrificed after 13 weeks of exposure, i.e., approximately 91 days. Females were sacrificed after 16 weeks, i.e. at weaning, approximately 120 days of exposure."

This report gives the results of some statistical analyses of the ammonium perchlocate experiment. The design of the experiment was to randomly assign rat parent pairs to one of four ammonium perchlocate dose groups. The doses were 0, .3, 3, and 30 (units unknown). Both parents were dosed 10 weeks before mating. Dosing of females continued through weaning or about age 21 days. One male and one female pup from each litter were sacrificed at age 21 days and TSH, T3, and T4 measurements were made.

The design of this experiment is a split-plot. The main plot treatment is the perchlocate dose which was applied to litters (since treatments were applied to the parents-mainly the mother) and the subplot "treatment" is gender. These designs are characterized by different mean square errors for evaluating different effects or classification variables in the experiment. Since three variables are measured on each pup, the proper analysis is a multivariate analysis of variance for a split-plot experiment. Essentially this is an analysis of the vector of three measurements from each pup.

The attached Table 1 gives the sample size, mean, and S.E. for each gender, dose, and variable combination. The means for the three variables are plotted in Figures 1 through 3. The multivariate analysis of variance results for the two main effects and the interaction effect are given in Table 2. All three effects are statistically significant ($p<.05$). The next step in the analysis is to do a univariate analysis of variance on each variable in order to understand the meaning of the significant multivariate effects. These latter analyses are for a spit-plot design. Since we are doing three analyses on one experiment, the Bonferroni adjustment to the p-values is made and are given under "Adjusted P" in each table. These adjusted p-values will be used to make conclusions from the experiment.

In the analysis of TSH, the dose by gender interaction is significant ($p=.002$) so a separate analysis of the dose effect only was done on each gender. The dose effect was not significant ($p=.420$) for females, but was significant ($p<.001$) for males. The conclusion for females is that there are no significant differences in TSH by dose. For males, Tukey's multiple comparison procedure was done on the dose means to determine which were different from each other. The conclusion for males is that dose 3 was significantly lower than doses 0 and 30 and no other differences are significant ($p<.05$).

The gender effect is the only significant one for T3 ($p=.049$). The mean of 108.4 (S.E.=1.5) for males is larger than the mean of 105.7 (S.E.=1.4) for females. The conclusion is that there are no dose effects on T3, but there is a small but significant gender effect.

No effect or interaction is significant for T4. The conclusion is that neither dose nor gender had a significant effect on T4.

Table 1

Means and S.E.s of each variable by gender and ammonium perchlorate dose

Gender	Ammonium perchlorate dose	Variable: TSH n	TSH Mean	TSH S.E.	T3 n	T3 Mean	T3 S.E.	T4 n	T4 Mean	T4 S.E.
Female	0	28	1.12	0.10	28	106	2.5	28	4.27	0.19
	.3	22	1.19	0.08	22	110	2.8	22	4.86	0.20
	3	25	1.14	0.07	25	109	2.7	25	4.32	0.16
	30	23	1.30	0.07	23	98	2.3	23	3.91	0.20
Male	0	27	1.24	0.09	27	106	1.9	27	4.40	0.20
	.3	21	0.94	0.07	21	111	3.6	21	4.61	0.21
	3	25	0.88	0.05	25	110	3.1	25	4.53	0.16
	30	23	1.27	0.08	23	107	3.3	23	4.53	0.23

Table 2

Multivariate analysis of variance of ammonium perchlorate data. Results for Wilks' Lambda statistic.

Source	D.F.	F	P
Dose	9, 226	2.29	.018
Gender	3, 89	5.69	.001
Dose x Gender	9, 217	3.67	<.001

Table 3

Analysis of variance of each variable in ammonium perchlorate experiment

Source	D.F.	Mean Square	F	P	Adjusted P
TSH					
Dose	3	.7510	3.13	.029	.087
Error 1	95	.2398			
Gender	1	.6243	10.85	.001	.004
Dose x Gender	3	.3745	6.51	<.001	.002
Error 2	91	.0576			
T3					
Dose	3	605.0	2.17	.097	.291
Error 1	95	279.0			
Gender	1	549.2	6.00	.016	.049
Dose x Gender	3	215.7	2.35	.077	.232
Error 2	91	91.6			
T4					
Dose	3	2.517	1.92	.132	.396
Error 1	95	1.314			
Gender	1	1.280	2.61	.109	.328
Dose x Gender	3	1.588	3.24	.026	.077
Error 2	91	.490			

Figure 1
TSH by dose and gender

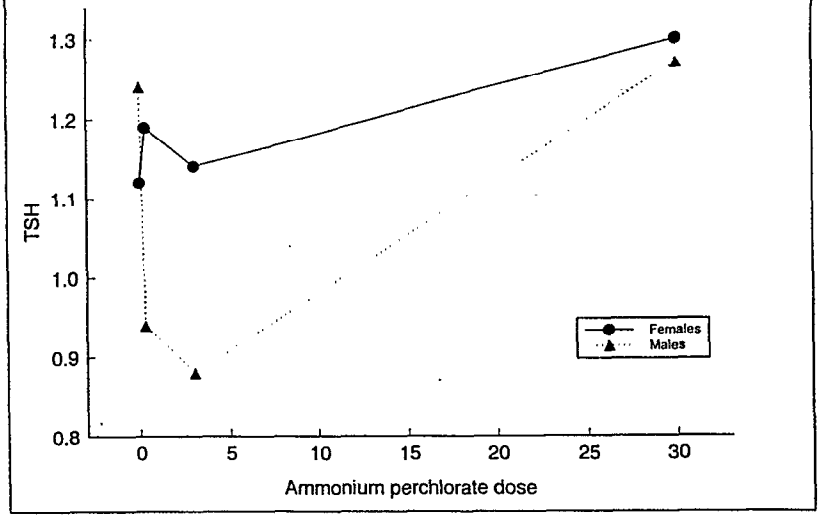


Figure 2
T3 by dose and gender

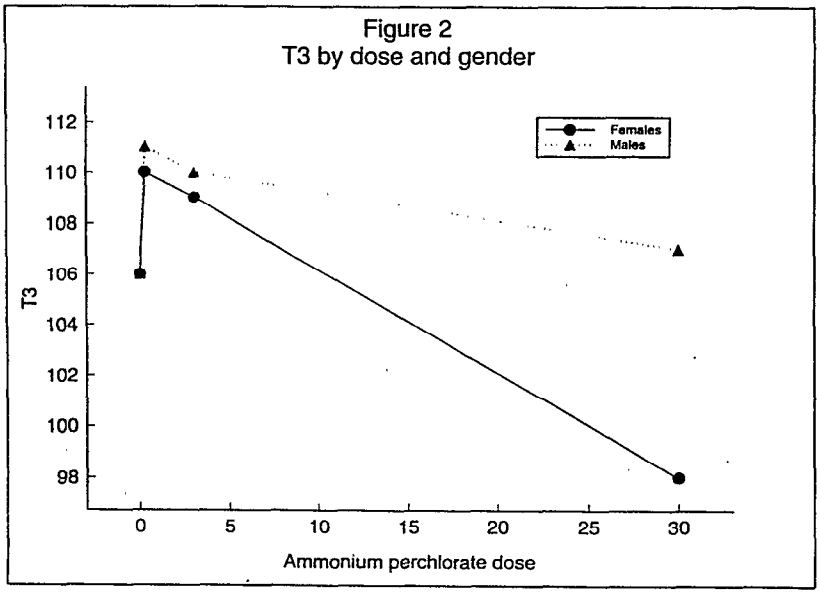
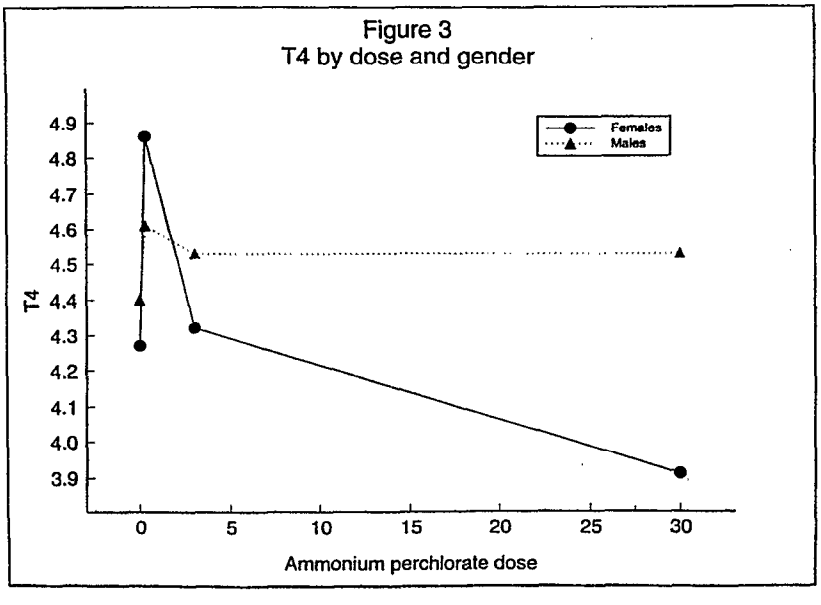


Figure 3
T4 by dose and gender



February 1, 1999 EPA Assessment Submission

Attachment #5
Analysis of Reproductive Parameters from the F1 Mating in Argus (1998b) 2-Generation Reproductive Study

A. Argus 1/15/99 Data Submission (York, 1999a)
B. EPA analysis (Clegg, 1999)

ATTENTION PANEL MEMBER(S):

ROCHELLE TYL

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
WASHINGTON, DC 20460

January 28, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Assimilation of F1 mating, estrous cyclicity and sperm measure results with P results

FROM: Eric D. Clegg, Ph.D. E. D. Clegg
National Center for Environmental Assessment (8623D)
Washington, DC

TO: Annie Jarabek
National Center for Environmental Assessment (MD-52)
Research Triangle Park, NC

I have reviewed the result tables on the F1 mating, estrous cyclicity and sperm measure results provided by Ray York of Argus Laboratories on January 15, 1999. The only statistically different result in the new data are in the fertility results where the control mating and pregnancy rates were significantly lower than the dosed groups. The values for the dosed groups were uniformly high. There was nothing remarkable in the results for the other parameters. The results with the P generation in mating and estrous cycle monitoring hinted at effects at 0.3 mg/kg, but those were not replicated with the F1 generation. Thyroid and ovarian weight data are not available yet for the F1. Thus, to this point, the F1 data are not supporting the existence of U-shaped dose-responses.

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
Telefax: (215) 443-8587

January 15, 1999

Joan Dollarhide
Toxicology Excellence for Risk Assessment (TERA)
4303 Hamilton Avenue
Cincinnati, Ohio 45223

Telephone: (606) 428-2744
Fax: (606) 428-3386

RE: Protocol 1416-001 - Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats

Dear Joan:

Attached is a copy of the audited individual and summary tables with the F1 generation sperm and estrous cycling data you requested. Please remember, these data could still change based on the final audit of the other study data.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Raymond G. York, Ph.D., DABT
Associate Director of Research
and Study Director

RGY:rgy
Enc.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D15 (PAGE 1): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS EXAMINED	N	30	30	30	27a
INCLUDED IN ANALYSES	N	29b	30	29b	27
NUMBER MOTILE	MEAN±S.D.	420.6 ± 158.4	400.4 ± 163.2	397.9 ± 186.0	449.0 ± 145.0
MOTILE PERCENT	MEAN±S.D.	77.2 ± 7.8	76.9 ± 8.1	76.4 ± 7.2	80.6 ± 5.7
STATIC COUNT (NONMOTILE)	MEAN±S.D.	116.1 ± 38.6	110.6 ± 39.7	114.6 ± 48.3	107.1 ± 45.4
TOTAL COUNT	MEAN±S.D.	536.6 ± 171.6	511.0 ± 181.3	512.5 ± 212.9	556.1 ± 170.6
SPERM COUNT	MEAN±S.D.	186.9 ± 59.7	200.9 ± 73.1	181.3 ± 53.9	179.0 ± 61.8
SPERM CONCENTRATION	MEAN±S.D.	10.9 ± 3.4	11.8 ± 4.3	10.6 ± 3.1	10.4 ± 3.6
SPERM DENSITY	MEAN±S.D.	1543.6 ± 520.8	1571.6 ± 536.1	1461.2 ± 438.8	1372.6 ± 444.6
SPERMATID COUNT	MEAN±S.D.	36.6 ± 15.6	35.6 ± 14.0	33.4 ± 9.4	29.6 ± 12.1
SPERMATID CONCENTRATION	MEAN±S.D.	2.1 ± 0.9	2.1 ± 0.8	1.9 ± 0.5	1.7 ± 0.8
SPERMATID DENSITY	MEAN±S.D.	125.0 ± 44.4	117.2 ± 46.2	109.3 ± 28.6	97.6 ± 41.2

a. Excludes values for rats that were found dead.

b. Excludes values for rats that had abnormal epididymides and testes.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D16 (PAGE 1): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS EXAMINED	N	30	30	30	27a
INCLUDED IN ANALYSES	N	29b	30	29b	27
NORMAL	MEAN±S.D.	189.5± 6.3	188.8± 4.9	190.1± 4.9	188.1± 5.4
PERCENT ABNORMAL	MEAN±S.D.	5.4± 3.1	5.6± 2.5	4.9± 2.4	5.9± 2.7
NO HOOK	MEAN±S.D.	0.2± 0.5	0.2± 0.6	0.1± 0.2	0.1± 0.4
EXCESSIVE HOOK	MEAN±S.D.	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.2
AMORPHOUS	MEAN±S.D.	0.0± 0.0	0.1± 0.2	0.0± 0.0	0.0± 0.2
PIN HEAD	MEAN±S.D.	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.2
DETACHED HEAD	MEAN±S.D.	7.9± 5.1	7.7± 4.1	6.9± 4.0	8.1± 5.0
NO HEAD	MEAN±S.D.	2.2± 1.6	2.6± 2.0	2.4± 1.4	2.8± 1.9
BANANA	MEAN±S.D.	0.0± 0.2	0.1± 0.2	0.1± 0.4	0.2± 0.8
COILED FLAGELLUM	MEAN±S.D.	0.0± 0.2	0.1± 0.2	0.0± 0.2	0.0± 0.0
BENT FLAGELLUM	MEAN±S.D.	0.0± 0.0	0.0± 0.0	0.0± 0.2	0.0± 0.2
BENT FLAGELLUM TIP	MEAN±S.D.	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
BROKEN FLAGELLUM	MEAN±S.D.	0.5± 0.8	0.5± 0.8	0.3± 0.7	0.4± 0.6

a. Excludes values for rats that were found dead.
b. Excludes values for rats that had abnormal epididymides and testes.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D26 (PAGE 1): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - F1 GENERATION MALE RATS

DOSAGE GROUP 1			0 (CARRIER) MG/KG/DAY						
RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM CONCENTRATION	SPERM DENSITY c	SPERMATID COUNT	SPERMATID CONCENTRATION	SPERMATID DENSITY
7001	192	74	69	261	153	9.4	1399.1	43	2.5	153.5
7002	438	76	142	580	96	5.6	826.5	48	2.8	182.1
7003	588	90	64	652	182	10.5	1339.6	26	1.5	90.3
7004d	0	0	6	6	2	0.1	41.3	16	0.9	279.7
7005	360	76	113	473	237	13.7	1722.5	34	2.0	120.9
7006	178	56	142	320	146	8.4	1422.0	26	1.5	100.5
7007	281	75	92	373	247	14.3	2205.2	33	1.9	107.4
7008	447	85	77	524	254	14.7	1800.9	27	1.6	97.2
7009	211	74	73	284	243	14.1	2466.4	33	1.9	112.8
7010	669	83	133	802	183	10.6	1470.5	29	1.7	97.4
7011	235	81	54	289	165	9.5	1391.5	29	1.7	107.8
7012	386	70	145	491	234	13.5	2338.1	35	2.0	110.2
7013	657	85	116	773	341	19.7	2644.5	4	0.2	12.6
5650	711	80	180	891	169	9.8	1433.6	33	1.9	117.1
7015	559	85	100	659	157	9.1	1316.4	21	1.2	77.0
7016	274	69	122	396	310	17.9	2591.7	26	1.5	95.2
7017	654	83	131	785	195	11.3	1509.2	37	2.1	135.5
7018	351	84	66	417	178	10.3	1418.5	33	1.9	122.7
7019	332	76	107	439	225	13.0	2148.1	27	1.6	115.0
7020	326	69	149	475	144	8.3	1031.6	59	3.4	177.8
7021	626	86	98	724	155	9.0	1042.7	20	1.2	69.7
7022	456	74	161	617	107	6.2	834.3	44	2.5	159.3
7023	464	87	71	535	190	11.0	1473.5	46	2.7	142.0
7024	625	79	166	791	159	9.1	1151.3	78	4.5	207.4
7025	347	73	128	475	125	7.2	1092.4	35	2.0	130.6
7026	289	78	82	371	169	9.8	1384.9	26	1.5	89.9
7027	490	85	85	575	248	14.3	1897.9	44	2.5	142.5
7028	452	73	169	621	187	10.8	1494.3	66	3.8	209.9
7029	309	62	193	502	101	5.8	936.4	33	1.9	128.2
7030	329	70	138	467	111	6.4	991.0	67	3.9	212.9

a. Sum of number motile and static count.

b. Sperm count used in the calculation of sperm density.

c. The sperm density is calculated by dividing the sperm count by the volume in the image area (34.3×10^{-5}), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) is multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table D24 for the weight of the left cauda epididymis, rounded to 3 decimal places) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

d. Rat 7004 had small and flaccid epididymides and testes; values excluded from group averages and statistical analyses.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D26 (PAGE 2): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - F1 GENERATION MALE RATS

DOSAGE GROUP 2			9.3 MG/KG/DAY						
RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM CONCENTRATION	SPERM DENSITY c	SPERMATID COUNT	SPERMATID CONCENTRATION	SPERMATID DENSITY
7031	162	72	62	224	176	10.2	1414.2	15	0.9	45.6
7032	455	81	105	560	171	9.9	1242.8	38	2.2	110.4
7033	192	71	79	271	199	11.5	1560.0	23	1.3	80.1
7034	475	71	194	669	131	7.6	1013.2	27	1.6	86.1
7035	326	78	92	418	226	13.1	1594.5	34	2.0	101.7
7036	314	80	79	393	224	13.0	1810.0	52	3.0	159.2
7037	311	78	87	398	286	16.5	2154.5	40	2.3	120.8
7038	537	77	161	698	256	14.8	2204.0	29	1.7	99.3
7039	277	68	129	406	329	19.0	2524.4	33	1.9	121.4
7040	438	80	108	546	216	12.5	1899.2	39	2.3	120.4
7041	212	70	89	301	199	11.5	1683.2	15	0.9	52.8
7042	436	90	48	484	292	16.9	2513.9	8	0.5	32.3
7043	423	78	117	540	265	15.3	2109.2	41	2.4	121.5
7044	523	92	48	571	205	11.9	1382.3	39	2.3	137.2
7045	620	82	133	753	179	10.4	1341.4	17	1.0	58.5
7046	236	63	136	372	293	17.0	2278.4	21	1.2	67.4
7047	339	85	58	397	325	18.8	2254.5	15	0.9	45.9
7048	222	79	59	281	84	4.9	843.7	39	2.3	144.7
7049	568	83	114	682	133	6.5	1089.6	44	2.5	180.0
7050	175	60	115	290	256	18.4	2005.9	51	3.5	189.2
7051	592	77	174	766	126	7.3	1088.0	38	2.2	130.1
7052	785	84	147	932	176	10.2	1309.8	39	2.3	128.8
7053	316	74	110	426	292	16.9	1968.9	54	3.1	182.5
7054	167	61	107	274	257	14.9	1891.7	53	3.1	152.7
7055	400	69	192	592	91	5.3	849.2	44	2.5	147.8
7056	517	80	131	648	121	7.0	1035.6	43	2.5	167.9
7057	419	85	72	491	100	5.8	747.5	49	2.8	162.5
7058	697	88	98	795	201	11.6	1498.5	25	1.4	73.6
7059	542	81	129	671	127	7.3	959.2	32	1.9	98.9
7060	345	70	146	491	112	6.5	892.5	61	3.5	192.0

a. Sum of number motile and static count.

b. Sperm count used in the calculation of sperm density.

c. The sperm density is calculated by dividing the sperm count by the volume in the image area (34.3×10^{-6}), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) is multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table D24 for the weight of the left cauda epididymis, rounded to 3 decimal places) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

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TABLE D25 (PAGE 3): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - F1 GENERATION MALE RATS

DOSAGE GROUP 3			3.0 MG/KG/DAY						
RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM CONCENTRATION	SPERM DENSITY c	SPERMATID COUNT	SPERMATID CONCENTRATION	SPERMATID DENSITY
7061	336	74	117	453	190	11.0	1531.0	32	1.9	96.9
7062	257	74	92	349	159	9.2	1398.0	26	1.5	83.3
7063	212	64	117	329	142	8.2	1229.8	18	1.0	65.8
7064	169	68	80	249	196	11.3	1723.3	43	2.5	141.8
7065	246	77	75	321	202	11.7	1240.6	38	2.2	105.9
7066	331	74	108	419	177	10.2	1296.2	24	1.4	90.3
7067	276	64	153	427	306	17.7	2445.2	24	1.4	77.6
7068	215	77	66	281	262	15.2	2469.7	27	1.6	100.8
7069	622	80	160	782	195	11.3	1532.8	34	2.0	118.2
7070d	0	0	8	8	6	0.3	92.8	0	0.0	0.0
7071	331	76	103	434	168	9.7	1717.2	24	1.4	83.3
7072	162	72	64	226	286	16.5	2224.0	38	2.2	114.5
7073	201	64	113	314	89	5.1	770.8	33	1.9	111.8
7074	558	82	125	683	168	9.7	1327.6	31	1.8	93.4
7075	834	91	83	917	138	8.0	1078.9	14	0.8	42.8
7076	274	87	42	316	164	9.5	1379.1	37	2.1	115.1
7077	338	80	86	424	144	8.3	980.1	45	2.6	148.1
7078	699	92	58	757	127	7.3	1043.7	48	2.8	139.1
7079	312	81	71	383	146	8.4	1456.3	32	1.9	120.6
7080	398	71	163	561	169	9.8	1800.8	47	2.7	149.2
7081	446	77	130	576	157	9.1	1170.5	28	1.6	96.9
7082	461	85	84	545	147	10.6	1436.6	27	1.6	95.6
7083	285	71	115	400	196	11.3	1633.9	42	2.4	160.3
7084	336	79	90	425	151	8.7	1456.0	29	1.7	103.0
7085	638	73	231	869	130	7.5	924.0	34	2.0	108.5
7086	408	81	95	503	190	11.0	1380.9	39	2.3	128.3
7087	346	78	100	446	239	13.8	1942.0	57	3.3	176.4
7088	425	68	200	625	155	9.0	1088.3	31	1.8	96.3
7089	706	79	185	889	315	18.2	2139.0	28	1.6	97.5
7090	742	77	217	959	150	8.7	957.9	40	2.3	109.3

a. Sum of number motile and static count.

b. Sperm count used in the calculation of sperm density.

c. The sperm density is calculated by dividing the sperm count by the volume in the image area (34.3×10^{-4}), multiplying by 2 (dilution factor) and multiplying by 10^{-4} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) is multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table D24 for the weight of the left cauda epididymis, rounded to 3 decimal places) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

d. Rat 7070 had small and flaccid epididymides and testes; values excluded from group averages and statistical analyses.

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TABLE D26 (PAGE 4): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - F1 GENERATION MALE RATS

DOSAGE GROUP 4			30.0 MG/KG/DAY						
RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM CONCENTRATION	SPERM DENSITY c	SPERMATID COUNT	SPERMATID CONCENTRATION	SPERMATID DENSITY
7091	355	70	153	508	124	7.2	1087.0	25	1.4	91.4
7092	245	76	76	321	295	17.1	2056.3	33	1.9	95.2
7093	540	72	210	750	190	11.0	1557.8	51	3.7	194.7
7094	296	84	58	354	271	15.7	1818.9	28	1.6	86.1
7095	425	78	120	545	190	11.0	1548.2	27	1.6	99.2
7096	249	79	65	314	224	13.0	1679.0	40	2.3	134.0
7097	471	82	102	573	213	12.3	1750.4	22	1.3	84.2
7098	647	81	154	801	120	6.9	1084.8	18	1.0	65.9
7099	467	89	56	523	135	7.8	1259.7	25	1.4	82.5
7100	333	86	56	389	245	14.2	2109.3	16	0.9	59.0
7101	397	85	69	466	215	12.4	1671.9	18	1.0	62.5
7102	249	80	62	311	273	15.8	2134.4	18	1.0	54.8
7103	431	78	125	556	228	13.2	1653.0	14	0.8	39.0
7104	638	88	83	721	132	7.6	1051.9	15	0.9	53.6
7105	562	83	114	676	103	6.0	721.4	18	1.0	61.9
7106	495	89	69	564	80	4.6	628.9	31	1.8	100.9
7107	212	75	70	282	136	7.9	936.7	4	0.2	11.9
7108	437	78	126	563	98	5.7	869.6	38	2.2	128.5
7109	444	68	207	651	140	8.1	1121.8	38	2.2	126.6
7110	836	85	144	980	203	16.4	1944.5	43	2.5	129.9
7111	FOUND DEAD ON DAY 131 POSTWEANING									
7112	605	81	146	751	159	9.2	1054.9	37	2.1	129.7
7113	FOUND DEAD ON DAY 95 POSTWEANING									
7114	537	75	182	719	152	8.8	1289.4	48	2.8	181.0
7115	FOUND DEAD ON DAY 82 POSTWEANING									
7116	467	84	89	556	139	8.0	1104.6	26	1.5	94.2
7117	293	75	100	393	195	11.3	1598.0	42	2.4	131.8
7118	552	87	83	635	225	13.0	1575.9	40	2.3	107.3
7119	508	87	79	587	114	6.6	774.1	44	2.5	127.7
7120	433	82	93	526	154	8.9	976.9	39	2.3	115.8

a. Sum of number motile and static count.

b. Sperm count used in the calculation of sperm density.

c. The sperm density is calculated by dividing the sperm count by the volume in the image area (34.3×10^{-4}), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) is multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table D24 for the weight of the left cauda epididymis, rounded to 3 decimal places) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

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TABLE D27 (PAGE 1): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - F1 GENERATION MALE RATS

ANIMAL NUMBER	NORMAL	NO HOOK	EXCESSIVE HOOK	AMORPHOUS	PIN HEAD	DETACHED HEAD	NO HEAD	BANANA	COILED FLAGELLUM	BENT FLAGELLUM	BENT FLAGELLUM TIP	BROKEN FLAGELLUM	PERCENT ABNORMAL
DOSAGE GROUP 1						0 (CARRIER) MG/KG/DAY							
7001	194	0	0	0	0	3	2	0	0	0	0	1	3.0
7002	168	2	0	0	0	25	3	0	0	0	0	2	16.0
7003	187	0	0	0	0	8	3	0	0	0	0	2	6.5
7004a	9	0	0	0	0	1	8	0	0	0	0	0	50.0
7005	196	0	0	0	0	4	0	0	0	0	0	0	2.0
7006	195	0	0	0	0	4	1	0	0	0	0	0	2.5
7007	192	0	0	0	0	4	3	0	0	0	0	1	4.0
7008	192	0	0	0	0	7	1	0	0	0	0	0	4.0
7009	181	1	0	0	0	10	7	0	0	0	0	1	9.5
7010	193	0	0	0	0	7	0	0	0	0	0	0	3.5
7011	177	0	0	0	0	20	3	0	0	0	0	0	11.5
7012	188	0	0	0	0	9	3	0	0	0	0	0	6.0
7013	195	0	0	0	0	3	2	0	0	0	0	0	2.5
5450	188	1	0	0	0	9	1	0	1	0	0	0	6.0
7015	192	1	0	0	0	4	3	0	0	0	0	0	4.0
7016	195	0	0	0	0	1	2	1	0	0	0	1	2.5
7017	187	1	0	0	0	8	3	0	0	0	0	1	6.5
7018	188	0	0	0	0	8	4	0	0	0	0	0	6.0
7019	186	1	0	0	0	7	3	0	0	0	0	3	7.0
7020	192	0	0	0	0	4	3	0	0	0	0	1	4.0
7021	190	0	0	0	0	9	1	0	0	0	0	0	5.0
7022	196	0	0	0	0	4	0	0	0	0	0	0	2.0
7023	196	0	0	0	0	4	0	0	0	0	0	0	2.0
7024	184	0	0	0	0	14	2	0	0	0	0	0	8.0
7025	189	0	0	0	0	9	2	0	0	0	0	0	5.5
7026	191	0	0	0	0	9	0	0	0	0	0	0	4.5
7027	184	0	0	0	0	12	4	0	0	0	0	0	8.0
7028	192	0	0	0	0	4	4	0	0	0	0	0	4.0
7029	190	0	0	0	0	9	1	0	0	0	0	0	5.0
7030	197	0	0	0	0	9	3	0	0	0	0	1	6.2

a. Rat 7004 had small and flaccid epididymides and testes; values excluded from group averages and statistical analyses.

PROTOCOL 3416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D27 (PAGE 2): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - F1 GENERATION MALE RATS

ANIMAL NUMBER	NORMAL	NO HOOK	EXCESSIVE HOOK	AMORPHOUS	PIN HEAD	DETACHED HEAD	NO HEAD	BANANA	COILED FLAGELLUM	BENT FLAGELLUM	BENT FLAGELLUM TIP	BROKEN FLAGELLUM	PERCENT ABNORMAL
DOSAGE GROUP 2						0.3 MG/KG/DAY							
7031	190	0	0	0	0	8	1	0	0	0	0	1	5.0
7032	183	0	0	0	0	14	2	0	0	0	0	1	8.5
7033	194	0	0	0	0	5	1	0	0	0	0	0	3.0
7034	191	1	0	0	0	3	5	0	0	0	0	0	4.5
7035	193	0	0	1	0	4	1	0	0	0	0	1	3.5
7036	190	0	0	0	0	6	3	0	1	0	0	0	5.0
7037	181	0	0	0	0	9	7	1	0	0	0	2	9.5
7038	192	0	0	0	0	5	3	0	0	0	0	0	4.0
7039	187	0	0	0	0	8	3	0	0	0	0	2	6.5
7040	189	0	0	0	0	9	1	0	1	0	0	0	5.5
7041	185	0	0	0	0	9	3	0	0	0	0	3	7.5
7042	196	0	0	0	0	3	1	0	0	0	0	0	2.0
7043	189	0	0	0	0	4	7	0	0	0	0	0	5.5
7044	190	0	0	0	0	8	1	0	0	0	0	1	5.0
7045	195	0	0	0	0	4	0	1	0	0	0	0	2.5
7046	186	2	0	0	0	7	4	0	0	0	0	1	7.0
7047	186	2	0	1	0	7	2	0	0	0	0	0	6.0
7048	182	0	0	0	0	13	5	0	0	0	0	0	9.0
7049	188	0	0	0	0	10	2	0	0	0	0	0	6.0
7050	189	1	0	0	0	8	2	0	0	0	0	0	5.5
7051	194	0	0	0	0	4	2	0	0	0	0	0	3.0
7052	191	0	0	0	0	6	3	0	0	0	0	0	4.5
7053	182	0	0	0	0	18	0	0	0	0	0	0	9.0
7054	193	0	0	0	0	5	1	0	0	0	0	1	3.5
7055	189	0	0	0	0	9	1	0	0	0	0	1	5.5
7056	191	0	0	0	0	6	3	0	0	0	0	0	4.5
7057	174	0	0	0	0	18	7	0	0	0	0	1	13.0
7058	192	0	0	0	0	4	4	0	0	0	0	0	4.0
7059	185	0	0	0	0	13	2	0	0	0	0	0	7.5
7060	195	0	0	0	0	3	1	0	0	0	0	1	2.5

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TABLE D27 (PAGE 3): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - P1 GENERATION MALE RATS

ANIMAL NUMBER	NORMAL	NO HOOK	EXCESSIVE HOOK	AMORPHOUS	PIN HEAD	DETACHED HEAD	NO HEAD	BANANA	COILED FLAGELLUM	BENT FLAGELLUM	BENT FLAGELLUM TIP	BROKEN FLAGELLUM	PERCENT ABNORMAL
DOSAGE GROUP 3						3.0 MG/KG/DAY							
7061	194	0	0	0	0	4	2	0	0	0	0	0	3.0
7062	190	1	0	0	0	2	3	0	1	0	0	3	5.0
7063	185	0	0	0	0	10	5	0	0	0	0	0	7.5
7064	188	0	0	0	0	8	3	0	0	0	0	1	6.0
7065	188	0	0	0	0	8	4	0	0	0	0	0	6.0
7066	193	0	0	0	0	4	2	1	0	0	0	0	3.5
7067	195	0	0	0	0	3	2	0	0	0	0	0	2.5
7068	191	0	0	0	0	5	4	0	0	0	0	0	4.5
7069	193	0	0	0	0	6	1	0	0	0	0	0	3.5
7070a	11	0	0	0	0	2	8	0	0	0	0	0	47.6
7071	191	0	0	0	0	6	2	0	0	1	0	0	4.5
7072	186	1	0	0	0	11	2	0	0	0	0	0	7.0
7073	195	0	0	0	0	4	1	0	0	0	0	0	2.5
7074	191	0	0	0	0	6	3	0	0	0	0	0	4.5
7075	197	0	0	0	0	2	1	0	0	0	0	0	1.5
7076	191	0	0	0	0	7	2	0	0	0	0	0	4.5
7077	173	0	0	0	0	20	7	0	0	0	0	0	13.5
7078	193	0	0	0	0	7	0	0	0	0	0	0	3.5
7079	188	0	0	0	0	10	2	0	0	0	0	0	6.0
7080	188	0	0	0	0	9	2	1	0	0	0	0	6.0
7081	185	0	0	0	0	11	3	0	0	0	0	1	7.5
7082	196	0	0	0	0	2	2	0	0	0	0	0	2.0
7083	191	0	0	0	0	6	3	0	0	0	0	0	4.5
7084	195	0	0	0	0	3	2	0	0	0	0	0	2.5
7085	190	0	0	0	0	8	1	1	0	0	0	0	5.0
7086	192	0	0	0	0	7	1	0	0	0	0	0	4.0
7087	190	0	0	0	0	5	3	0	0	0	0	2	5.0
7088	185	0	0	0	0	14	1	0	0	0	0	0	7.5
7089	184	0	0	0	0	10	4	1	0	0	0	1	8.0
7090	195	0	0	0	0	3	2	0	0	0	0	0	2.5

a. Rat 7070 had small and flaccid epididymides and testes; values excluded from group averages and statistical analyses.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D27 (PAGE 4): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - F1 GENERATION MALE RATS

ANIMAL NUMBER	NORMAL	NO HOOK	EXCESSIVE HOOK	AMORPHOUS	PIN HEAD	DETACHED HEAD	NO HEAD	BANANA	COILED FLAGELLUM	BENT FLAGELLUM	BENT FLAGELLUM TIP	BROKEN FLAGELLUM	PERCENT ABNORMAL
DOSAGE GROUP 4						30.0 MG/KG/DAY							
7091	190	0	0	0	0	8	2	0	0	0	0	0	5.0
7092	174	0	0	0	0	22	3	0	0	0	0	1	13.0
7093	182	0	0	0	0	17	1	0	0	0	0	0	9.0
7094	194	0	0	0	0	6	0	0	0	0	0	0	3.0
7095	189	0	0	0	0	7	4	0	0	0	0	0	5.5
7096	190	0	0	1	0	4	4	0	0	0	0	1	5.0
7097	184	0	0	0	0	11	3	0	0	0	0	2	8.0
7098	193	0	0	0	1	6	0	0	0	0	0	0	3.5
7099	192	0	0	0	0	4	4	0	0	0	0	0	4.0
7100	192	0	0	0	0	7	1	0	0	0	0	0	4.0
7101	188	2	0	0	0	0	6	4	0	0	0	0	6.0
7102	196	0	0	0	0	1	3	0	0	0	0	0	2.0
7103	188	0	0	0	0	8	4	0	0	0	0	0	6.0
7104	181	0	0	0	0	13	6	0	0	0	0	0	9.5
7105	183	0	0	0	0	11	5	0	0	0	0	1	8.5
7106	194	0	0	0	0	2	3	0	0	0	0	1	3.0
7107	190	0	0	0	0	7	2	0	0	0	0	1	5.0
7108	188	0	0	0	0	5	6	0	0	0	0	1	6.0
7109	181	0	0	0	0	10	5	0	0	0	0	1	8.1
7110	188	1	0	0	0	8	3	0	0	0	0	0	6.0
7111	FOUND DEAD ON DAY 131 POSTWEANING												
7112	192	0	0	0	0	6	2	0	0	0	0	0	4.0
7113	FOUND DEAD ON DAY 95 POSTWEANING												
7114	193	0	0	0	0	5	1	0	0	0	0	1	3.5
7115	FOUND DEAD ON DAY 82 POSTWEANING												
7116	188	0	0	0	0	8	3	0	0	0	0	1	6.0
7117	195	0	0	0	0	5	0	0	0	0	0	0	2.5
7118	191	0	0	0	0	8	0	1	0	0	0	0	4.5
7119	181	0	0	0	0	17	2	0	0	0	0	0	9.5
7120	181	0	1	0	0	12	4	0	0	1	0	1	9.5

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E22 (PAGE 1): ESTROUS CYCLING, MATING AND FERTILITY - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
PRECOHABITATION ESTROUS CYCLING					
RATS EVALUATED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	29a	30	30
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	5.0 ± 0.8	4.8 ± 0.8	4.9 ± 0.7	4.9 ± 1.0
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	3	3	0	3
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	0	0	0	0

a. Excludes values for rat 7253, which was moribund sacrificed on day 62 postweaning (day 3 of estrous cycling).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E22 (PAGE 2): ESTROUS CYCLING, MATING AND FERTILITY - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS IN COHABITATION	N	30	29a	30	30
DAYS IN COHABITATION b	MEAN±S.D	2.9 ± 2.8	3.2 ± 2.6	2.9 ± 1.6	2.4 ± 1.2 [29]c
RATS THAT MATED	N(%)	29(96.7)	28(96.6)	30(100.0)	30(100.0)
FERTILITY INDEX d	N/N (%)	21/29 (72.4)	27/28** (96.4)	28/30** (93.3)	27/30** (90.0)
RATS WITH CONFIRMED MATING DATES	N	29	28	30	29c
RATS MATING c,e					
DAYS 1- 7	N(%)	28(96.6)	28(100.0)	30(100.0)	29(96.7)
DAYS 8-14	N(%)	1(3.4)	0(0.0)	0(0.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	21/30 (70.0)	27/29** (93.1)	28/30** (93.3)	27/30** (90.0)

[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 7253, which was moribund sacrificed on day 62 postweaning (day 3 of estrous cycling).
b. Restricted to rats with a confirmed mating date and rats that did not mate.
c. Excludes values for dam 7307, which was cohabited with a second male rat.
d. Number of pregnancies/number of rats that mated.
e. Restricted to rats with a confirmed mating date.
** Significantly different from the carrier group value ($p \leq 0.01$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E44 (PAGE 1): ESTROUS CYCLING AND DAYS IN COHABITATION - INDIVIDUAL DATA - F1 GENERATION FEMALE RATS

RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION	RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION
DOSAGE GROUP 1			0 (CARRIER) MG/KG/DAY		
7201	5	3	7216	5	1
7202	4a	1	7217	5	1
7203	6	4	7218	5	2
7204	5	1	7219	5	1
7205	3a	3	7220	4	2
7206	6	4	7221	4	1
7207	5	5	7222	5	2
7208	5	9	7223	5	1
7209	4	2	7224	5	3
7210	4a	1	7225	6	1
7211	1	1	7226	6	1
7212	5	1	7227	5	3
7213	5	3	7228	6	4
7214	6	1	7229	5	3
7215	6	5	7230	6	14b

a. Six or more consecutive days of diestrus were observed.
b. Mating not confirmed.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E44 (PAGE 2): ESTROUS CYCLING AND DAYS IN COHABITATION - INDIVIDUAL DATA - F1 GENERATION FEMALE RATS

RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION	RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION
DOSAGE GROUP 2			0.3 MG/KG/DAY		
7231	3a	6	7246	5	1
7232	5	6	7247	5	2
7233	6	4	7248	5	2
7234	5	2	7249	5	1
7235	5	2	7250	3a	3
7236	5	3	7251	4	3
7237	3	1	7252	5	4
7238	5	1	7253b		
7239	5	3	7254	5	1
7240	5	1	7255	3a	7
7241	5	2	7256	5	3
7242	6	4	7257	5	2
7243	5	14c	7258	5	4
7244	5	3	7259	5	4
7245	5	3	7260	5	2

a. Six or more consecutive days of diestrus were observed.
b. Rat 7253 was moribund sacrificed on day 62 postweaning (day 3 of estrous cycling).
c. Mating not confirmed.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E44 (PAGE 3): ESTROUS CYCLING AND DAYS IN COHABITATION - INDIVIDUAL DATA - F1 GENERATION FEMALE RATS

RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION	RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION
DOSAGE GROUP 3			3.0 MG/KG/DAY		
7261	5	1	7276	5	2
7262	6	4	7277	5	1
7263	5	7	7278	5	2
7264	5	4	7279	5	1
7265	4	2	7280	5	6
7266	6	1	7281	4	6
7267	4	2	7282	5	3
7268	5	1	7283	5	3
7269	3	2	7284	5	2
7270	5	3	7285	5	2
7271	5	3	7286	6	4
7272	6	4	7287	5	2
7273	3	5	7288	5	3
7274	5	3	7289	5	3
7275	5	1	7290	5	3

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E44 (PAGE 4): ESTROUS CYCLING AND DAYS IN COHABITATION - INDIVIDUAL DATA - F1 GENERATION FEMALE RATS

RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION	RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION
DOSAGE GROUP 4			30.0 MG/KG/DAY		
7291	6	1	7306	6	4
7292	4a	3	7307	6	8b
7293	3	1	7308	5	4
7294	6	4	7309	6	4
7295	4	1	7310	5	2
7296	6	2	7311	5	2
7297	6	4	7312	3	3
7298	6	2	7313	4	1
7299	4a	2	7314	4	1
7300	4	2	7315	5	1
7301	5	6	7316	5	3
7302	5	3	7317	5	2
7303	4	3	7318	5	2
7304	3a	3	7319	4	2
7305	5	2	7320	7	1

a. Six or more consecutive days of diestrus were observed.
c. Dam 7307 was cohabited with a second male rat; values excluded from group averages and statistical analyses.

February 1, 1999 EPA Assessment Submission

Attachment #6
Sheep Red Blood Cell (SRBC) Assay in 90-day Studies

A. Keil 1/23/99 Data Submission
B. EPA analysis (Smialowicz, 1999)

ATTENTION PANEL MEMBER(S):

KIMBER WHITE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
National Health and Environmental Effects Research Laboratory
Experimental Toxicology Division
Research Triangle Park, NC 27711

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

DATE: January 28, 1999

FROM: Ralph J. Smialowicz (MD-92) RJ Smialowicz

TO: Annie Jarabek (MD-52)
National Center for Environmental Assessment

SUBJECT: Review of 90-Day Ammonium Perchlorate Exposure on the Antibody Response to SRBC in Mice

As indicated in the external review draft of the NCEA document entitled *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information,* an evaluation of the potential effects of ammonium perchlorate on humoral immunity was not performed as part of the original immunotoxicity testing protocol. This raised concern that a significant component of the immune system was not assessed in perchlorate-exposed animals. Consequently, the sponsor and contract laboratory agreed to perform 14-day and 90-day studies in which the antibody response to sheep red blood cells (SRBC) would be determined.

Results of a 90-day study were received on January 23,1999. In this study, B6C3F1 female mice, 12 mice per group, were exposed to ammonium perchlorate (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 90 days. Mice where immunized intravenously with SRBC on day 75. Serum was collected on day 79 (4 days post-immunization) and on day 90 (15 days post-immunization), and the SRBC-specific IgM and IgG antibody levels were determined using an enzyme-linked immunosorbent assay (ELISA) "based on a protocol provided by L. Temple of the Medical College of Virginia". Analysis of the ELISA data, which was expressed as the O.D. 50, indicated that neither the IgM nor IgG titers were affected by ammonium perchlorate exposure. In the report, the contract laboratory indicated limitations which were the following: 1) a kinetic study to determine the day of peak levels of IgM and IgG was not performed; and 2) since specialized software (e.g., Softmax®) was not available, serum antibody titers were calculated as the O.D. 50 or midpoint "as described by a SOP provided by L. Temple", rather than the conventional "titer to achieve 0.5 O.D.".

The results of a 14-day exposure study on SRBC-specific antibody responses in mice is expected on February 3, 1999. In addition, because of concern expressed in the external review draft about the infectivity data (i.e., *L. monocytogenes* challenge model) additional studies are currently in progress. The expected due date for the report of these data is June 1, 1999.

SRBC Specific Serum IgM or IgG Determination after Exposure to Ammonium Perchlorate for 90 Days

Submitted by Deborah Keil, PhD
Medical University of South Carolina
January 23, 1999

Animals and Ammonium Perchlorate Exposure: B6C3F1 female mice aged 8-10 weeks were exposed to ammonium perchlorate (AP) (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 90 days. A total of 60 mice with 12 animals per treatment group were used to determine specific IgM and IgG levels after immunization with sRBC. Animals were housed in an AAALAC accredited facility and provided water (with and without AP) and mouse chow ad libidum.

Immunization: Mice were immunized with sheep red blood cells (sRBC) ($1x10^8$ total cells) by intravenous tail injection on day 75. Serum was collected on day 79 (4 days post challenge) and day 90 (15 days post challenge) to determine specific IgM or IgG sRBC antibody levels, respectively. A semi-quantitative ELISA detected levels of specific IgM or IgG sRBC antibody in serially diluted serum (1:20, 1:40, 1:80, 1:160, 1:320). A SOP based on a protocol provided by L. Temple of the Medical College of Virginia was used.

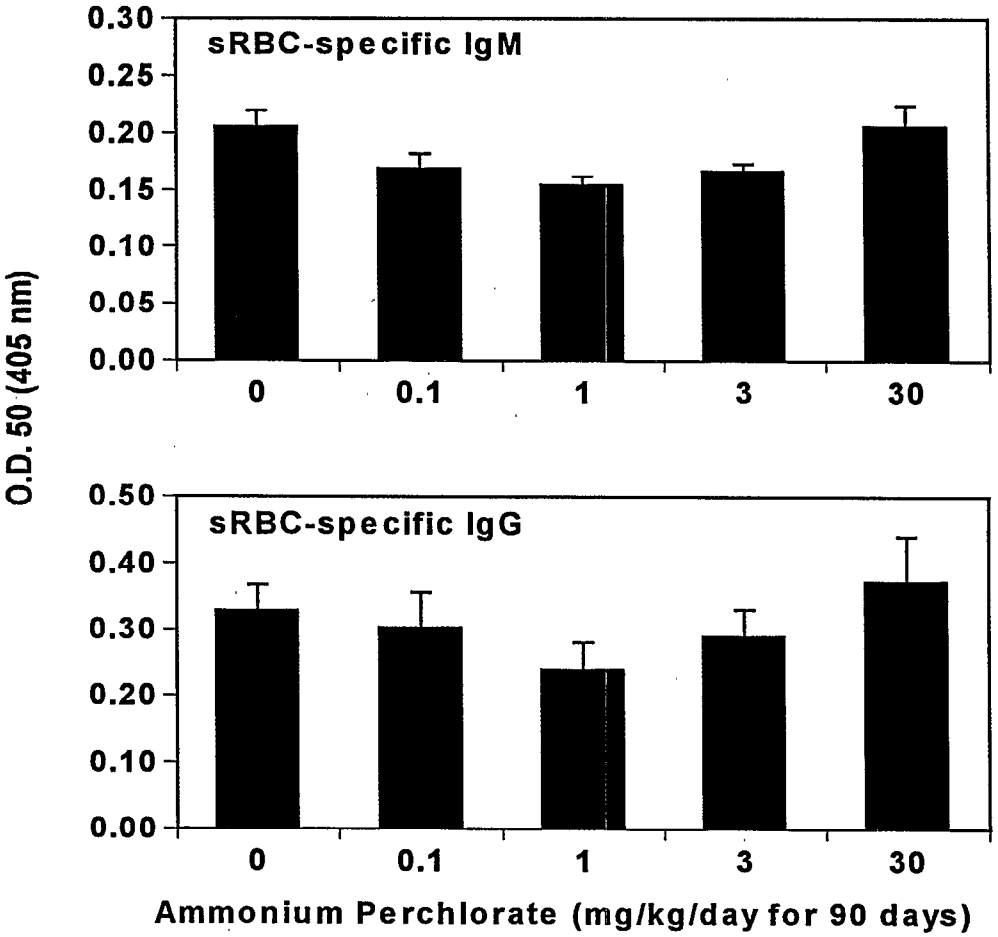
Optimization of the ELISA: Optimization of the ELISA was performed prior to testing the serum samples to establish the appropriate titer of sRBC membrane coating antigen (1μg/ml) and the secondary antibody dilution (1: 5,000 for IgM and 1:7,500 for IgG). In addition, pooled serum samples from controls were used in the optimization. Controls for non-specific binding were included and were less than 0.070 O.D. (405 nm) in both the optimization and testing ELISAs.

Data Analysis:
Analysis of sRBC specific IgG serum titers were analyzed as described in a SOP provided by Louise Temple of the Medical College of Virginia. The average absorbance unit values of the replicates for each dilution of the test serum were calculated. Background in the ELISA was subtracted from these values. Five consecutive average absorbance values versus log base 2 of the dilution of the serum were plotted. The best-fit linear line was calculated in an Excel spreadsheet by determining the value for the slope and intercept. Log base 2 of the titer was considered the independent variable and O.D. was considered the dependent variable. In this experiment, the absorbance at the mid-point of the 5 serial dilutions was 1:80 (log base 2 (80) = 6.3219). Using the equation for the best-fit line, the O.D. 50 (absorbance at mid-point 1:80) was calculated for each animal.

Results:
No significant differences were observed in any of the AP treatment groups as compared to controls for specific IgM or IgG levels after immunization with sRBC. This was determined by using the calculated O.D.50 for each sample and performing an analysis of variance with Tukey's pairwise comparisons ($p<0.05$). Refer to graphs and statistical analysis that have been included in this report.

Limitations: A time course to determine the peak levels of IgM or IgG after sRBC immunization in B6C3F1 female mice was not performed in this study. However, bleeding times (day 4 for IgM and day 15 for IgG) have been previously used and reported in the literature (Holsapple, et al, 1984).
In addition, these data may be analyzed by additional methods to include expression of the "serum titer to achieve 0.5 O.D." At this time, the data manipulation involved to determine the "serum titer to achieve 0.5 O.D." has been laborious and time-consuming, particularly when specialized software (i.e., Softmax) is not available to produce specialized graphs and corresponding equations for each of the 120 samples. Consequently, I have submitted the calculated O.D. 50 as described by a SOP provided by L. Temple.

Serum IgM or IgG Levels after sRBC Challenge During a 90-Day Exposure to Ammonium Perchlorate



Adult B6C3F1 female mice were exposed to ammonium perchlorate (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 90 days. On day 75, animals were immunized by i.v. tail injection with sRBC (1×10^8 cells). Following the immunization, animals were bled on day 79 (4 days post challenge) and day 90 (15 days post challenge) to obtain serum for detection of specific IgM or IgG respectively. Detection of specific IgM or IgG was performed using an ELISA based on a protocol provided by L. Temple at the Medical College of Virginia. The O.D. 50 was determined for both IgM and IgG. Each of the above graphs represent the means and standard errors of a total of 59 mice. No significant differences were observed in any of the treatment groups as compared to controls using analysis of variance and Tukey's pairwise comparisons ($p<0.05$).

Statistics

The calculated O.D. 50 for each of the treatment groups was compared to controls ($p<0.05$). A total of 59 serum samples from independently challenged mice were analyzed for both IgG and IgM.

One-way Analysis of Variance 90d IgG

Analysis of Variance

Source	DF	SS	MS	F	P
C4	4	0.1163	0.0291	0.92	0.458
Error	54	1.7020	0.0315		
Total	58	1.8183			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
0.0	12	0.3270	0.1413	(----------*---------)
0.1	11	0.3008	0.1827	(----------*----------)
1.0	12	0.2374	0.1499	(----------*---------)
3.0	12	0.2880	0.1522	(---------*---------)
30.0	12	0.3708	0.2424	(---------*---------)

Pooled StDev = 0.1775 — 0.20 — 0.30 — 0.40

Tukey's pairwise comparisons

Family error rate = 0.0500

Individual error rate = 0.00668

Critical value = 3.99

Intervals for (column level mean) - (row level mean)

	0.0	0.1	1.0	3.0
0.1	-0.1829 0.2353			
1.0	-0.1149 0.2941	-0.1457 0.2725		
3.0	-0.1655 0.2435	-0.1963 0.2219	-0.2551 0.1539	
30.0	-0.2483 0.1607	-0.2791 0.1391	-0.3379 0.0711	-0.2873 0.1217

Descriptive Statistics 90d IgG

Variable	N	N*	Mean	Median	TrMean	StDev
C6	12	0	0.3270	0.2940	0.3222	0.1413
C7	11	1	0.3008	0.2350	0.2908	0.1827
C8	12	0	0.2374	0.2050	0.2210	0.1499
C9	12	0	0.2880	0.2555	0.2728	0.1522
C10	12	0	0.3708	0.2925	0.3431	0.2424

Variable	SE Mean	Minimum	Maximum	Q1	Q3
C6	0.0408	0.1500	0.5520	0.2002	0.4737
C7	0.0551	0.0470	0.6450	0.1450	0.4330
C8	0.0433	0.0430	0.5960	0.1685	0.3143
C9	0.0439	0.1260	0.6020	0.1563	0.4192
C10	0.0700	0.1480	0.8710	0.1670	0.5853

One-way Analysis of Variance 90d IgM

Analysis of Variance

Source	DF	SS	MS	F	P
treatmen	4	0.02701	0.00675	3.13	0.022
Error	54	0.11640	0.00216		
Total	58	0.14341			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
0.0	12	0.20442	0.05200	(--------*--------)
0.1	11	0.16827	0.04506	(--------*--------)
1.0	12	0.15342	0.02899	(--------*--------)
3.0	12	0.16608	0.02613	(--------*--------)
30.0	12	0.20508	0.06714	(--------*--------)

Pooled StDev = 0.04643 0.150 0.180 0.210

Tukey's pairwise comparisons

Family error rate = 0.0500

Individual error rate = 0.00668

Critical value = 3.99

Intervals for (column level mean) - (row level mean)

	0.0	0.1	1.0	3.0
0.1	-0.01853 0.09082			
1.0	-0.00248 0.10448	-0.03982 0.06953		
3.0	-0.01514 0.09181	-0.05249 0.05687	-0.06614 0.04081	
30.0	-0.05414 0.05281	-0.09149 0.01787	-0.10514 0.00181	-0.09248 0.01448

Descriptive Statistics 90d IgM

Variable	N	N*	Mean	Median	TrMean	StDev
c	12	0	0.2044	0.2080	0.2014	0.0520
0.1	11	1	0.1683	0.1560	0.1649	0.0451
1	12	0	0.15342	0.15050	0.15030	0.02899
3	12	0	0.16608	0.16350	0.16580	0.02613
30	12	0	0.2051	0.2085	0.2083	0.0671

Variable	SE Mean	Minimum	Maximum	Q1	Q3
c	0.0150	0.1400	0.2990	0.1535	0.2415
0.1	0.0136	0.1020	0.2650	0.1450	0.1880
1	0.00837	0.12400	0.21400	0.12625	0.17100
3	0.00754	0.11700	0.21800	0.14825	0.18075
30	0.0194	0.0890	0.2890	0.1410	0.2682

February 1, 1999 EPA Assessment Submission

Attachment #7
Interim Thyroid Histopathology in Mice (Control and High Dose) from Keil et al. (1998) Immunotoxicity Studies

A. Warren 1/13/99 Data Submission
B. EPA analysis (Jarabek, 1999)

ATTENTION PANEL MEMBER(S):

TOM ZOELLER
SUSAN PORTERFIELD

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
RESEARCH TRIANGLE PARK, NC 27711

February 1, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Review of Interim Pathology Report in Mice from 90-day Immunotoxicity Studies

FROM: Annie M. Jarabek
National Center for Environmental Assessment
RTP (MD-52)

TO: EPA Perchlorate Health Assessment Team

I have reviewed the interim histopathology report received on January 13, 1999 for the mice from the immunotoxicity studies ongoing at the Medical Center of South Carolina (Warren, 1999). These are reported for two 90-day experiments ("A" and "D") and only for the control and high-dose groups. Thus, this analysis is preliminary and limited but nevertheless worthwhile to include at this time since it may add some perspective on interspecies sensitivity. The report is attached.

Three histologic sections (A,B,C) from different levels of the thyroid gland were prepared and submitted for potential histopathologic assessment. Initially, all sections were examined to select the best single section for detailed evaluation. For consistency in the selection of the region of thyroid gland for the detailed evaluation, only sections of the thyroid tissue that contained parathyroid gland were used, when possible. If parathyroid gland was not present, the specimen with the largest area of thyroid gland was used. The study pathologist did not read the slides blind, but rather as he notes in the E-mail attached to the report, read the control and high dose specimens to detect a putative morphologic alteration and to characterize the full range of the alterations. Although I understand these points, no mention of a second pathologist to provide QA (per typical NTP SOP) on the study was mentioned. I expect the issue that we have already raised regarding the lack of QA or blind assessment will be resolved in the disposition of the decision regarding a potential pathology working group (PWG) of all the thyroid histopathology, so that I will not belabor the point herein.

In both 90-day experiments ("A" and "D"), the incidence of lesions induced by treatment were 0 in the control and 100% in the 30 mg/kg-day group. Lesions consistent with our proposed mode-of-action were observed, including: colloid depletion, congestion, hypertrophy. Mean values for these lesions are given but the

severity range was not provided. The majority of follicles tended to be smaller (a few exceptions on the periphery) with less colloid. The nuclear to cytoplasmic ratio of the follicular cells was usually 1.5 to 2.0.

These lesions in mice are consistent with those seen in the other species tested and with the proposed mode-of-action for the assessment model. Quantitative interspecies comparison is precluded at this time due to the lack of completed histopathology at the other doses. The Caldwell et al. (1995) study in rats is the only one that tested as high as approximately 22 mg/kg-day, but the difference in severity ratings and lack of statistics for both reports prevents further analysis. In the rabbit developmental study, histopathology was observed at the 30 mg/kg-day dose and this was not the lowest observed effect level. The best data for comparison may be the pending histopathology in the adults of the 2-generation reproductive study in rats, since there was a 30 mg/kg-day testing dose.

In conclusion, this preliminary analysis suggests that the mode-of-action is similar in mice, rabbits and rats. Quantitative interspecies comparison awaits dose-response data in the mice (i.e., histopathology for the remaining dose groups) and possibly a systematic pathology working group (PWG) evaluation of all the histopathology data once they are available.

Attachment

TERRA, Inc.
Toxicology, Ecology, Research, and Risk Assessment

January 13, 1999

Annie Jarabek
NCEA National Center for Environmental Assessment
3210 Highway 54 Catawba Bldg.
RTP Durham, NC 27709-

RE:
Our Case File: MUSC-6872

Dear Ms. Jarabek:

Dave Mattie asked that I send you a copy of the interim pathology report prepared in relation to the perchlorate research effort ongoing at the Medical University of South Carolina. Although my involvement in the research project has been minimal since submitting the grant proposal, as a consultant I have had to stay informed on the issue. I congratulate you and your colleagues for your success in tackling a complex subject in such a systematic and expeditious fashion. I will forward the pathology analysis of the remaining dose groups to you in the near future. Please feel free to call me with your questions or concerns.

Best Regards,

Alan Warren
TERRA, INC.

Mahan Station • 1709 Mahan Drive, Suite E • Tallahassee, Florida 32308 • Phone (850) 309-1330 • Fax (850) 309-1336

John R. Latendresse, D.V.M., Ph.D.
Diplomate of the American College of Veterinary Pathologists

Phone 870-543-7404
E-mail jlatendresse@nctr.fda.gov

Interim Pathology Report
Histopathologic Effects of Ammonium Perchlorate in Thyroid Gland of Mice

Methods

Eight to nine week old male B6C3F1 mice were administered ammonium perchlorate in drinking water for 90 days at 0, 0.1, 1.0, 3.0, and 30 mg/kg/day in two different studies (Studies A and D). For inclusion in this report, only the control and high dose groups from each study were examined. Three histologic sections (A, B, and C) from different levels of the thyroid gland were prepared and submitted for potential histopathologic assessment. Initially, all sections were examined to select the best single section for a detailed evaluation. For consistency in the selection of the region of thyroid gland for the detailed evaluation, only sections of thyroid tissue that contained parathyroid gland were used, when possible. If parathyroid gland was not present, the specimen with the largest area of thyroid gland was used.

Results and Discussion

Morphologies by anatomical site and individual animal are given in the Histopathology Databases (Tables 1 and 2). Thyroid glands from control mice were essentially normal. The follicles were variably sized with complements of relatively large, medium and small colloid-filled lumens. The height of the follicular epithelium was mostly low to medium cuboidal, and the nuclear to cytoplasmic ratio was usually one or less. The cytoplasm of the follicular cells often contained abundant small vacuoles.

The incidence of lesions induced by treatment with ammonium perchlorate is given in the tables 3 and 4. In the 30 mg/kg/day group, although a few peripheral follicles were large with abundant colloid in their lumens, the majority of the follicles tended to be smaller on the average with less colloid compared to controls. Both the inter- and intrafollicular capillaries were mildly congested diffusely, distinguishing them from those of the control thyroid glands. The mildly hypertrophied follicular epithelium was characteristically high cuboidal to low columnar. The nuclear to cytoplasmic ratio of the follicular cells was usually 1.5 to 2. The follicular cells often contained clear perinuclear halos, but the distinct pattern of vacuolization observed in the control group was absent.

Table 3. Study A
Incidence (%) of Thyroid Gland Lesions in Mice Exposed to Ammonium Perchlorate

Anatomical Site	Morphology	Dose (mg/kg/day) 0	30
Thyroid follicle	Colloid depletion	0/6 (0)	6/6 (100) [2]*
Capillary	Congestion	0/6 (0)	6/6 (100) [2]
Epithelium, follicular	Hypertrophy	0/6 (0)	6/6 (100) [2]

- [Mean severity]

Table 4. Study D
Incidence (%) of Thyroid Gland Lesions in Mice Exposed to Ammonium Perchlorate

Anatomical Site	Morphology	Dose (mg/kg/day) 0	30
Thyroid follicle	Colloid depletion	0/5 (0)	5/5 (100) [2]*
Capillary	Congestion	0/6 (0)	5/5 (100) [1.8]
Epithelium, follicular	Hypertrophy	0/6 (0)	5/5 (100) [1.8]

- [Mean severity]

Inhibition of iodide uptake by the thyroid follicular epithelium has been reported as the mechanism of action of ammonium perchlorate in the rat thyroid gland. Iodination of tyrosine residues of thyroglobulin is one of the essential steps in the production of T3 and T4 (thyroxin). Decreased synthesis of T4 and T3 results in lowered serum concentration that triggers the synthesis and release of TSH from the anterior pituitary gland. TSH receptor activation of cyclic AMP intracellular signaling culminates in hypertrophy of the follicular epithelium. Epithelial hypertrophy, colloid depletion, and the appearance of increased blood flow to the thyroid gland observed in the mice in these studies are consistent with persistent TSH stimulation secondary to deficient production of T3 and/or thyroxin. These observations support a hypothesis of a similar mechanism of action of ammonium perchlorate in the thyroid gland of the mouse that has been shown in the rat.

John R. Latendresse
Diplomate, College of Veterinary Pathologists

Principle Investigator: A. Warren, Ph.D.

Table 1
Ammonium Perchlorate
Histopathology Database

Pathologist: J.R. Latendresse, D.V.M., Ph.D.
Diplomate, ACVP

Study ID	Dose mg/kg/day	Animal ID	Slide ID	Site	Diagnosis	Severity	Remarks
A	0	1	A	thyroid gland	essentially normal tissue		Follicles are variably sized. Follicular epithelium is low to medium cuboidal with the cytoplasmic to nuclear ratio usually equal to or less than 1. Cytoplasm is often vacuolated.
A	0	2	A	thyroid gland	essentially normal tissue		
A	0	4	A	thyroid gland	essentially normal tissue		
A	0	5	A	thyroid gland	essentially normal tissue		
A	0	6	C	thyroid gland	essentially normal tissue		
A	0	3	A	thyroid gland	thyroglossal duct cyst	2	Incidental congenital cyst commonly formed postnatally due to accumulation of proteinaceous fluid in thyroglossal duct remnant.
A	30	28	C	thyroid follicle	colloid depletion	2	
A	30	29	A	thyroid follicle	colloid depletion	2	
A	30	30	A	thyroid follicle	colloid depletion	2	
A	30	25	A	thyroid follicle	colloid depletion	2	
A	30	26	A	thyroid follicle	colloid depletion	2	
A	30	27	A	thyroid follicle	colloid depletion	2	
A	30	25	A	capillary	congestion	2	Inter- and intrafollicular capillaries are prominantly dilated and filled with erythrocytes.
A	30	26	A	capillary	congestion	2	
A	30	27	A	capillary	congestion	2	
A	30	28	C	capillary	congestion	2	
A	30	29	A	capillary	congestion	2	
A	30	30	A	capillary	congestion	2	
A	30	25	A	epithelium, follicular	hypertrophy	2	Follicles are variably sized. Height of the follicular epithelium is usually high cuboidal to low columnar. Area of follicular cytoplasm is usually 1.5 to 2x greater than controls making cytoplamic to nuclear ratio about 1.5 to 2.
A	30	26	A	epithelium, follicular	hypertrophy	2	
A	30	27	A	epithelium, follicular	hypertrophy	3	
A	30	28	C	epithelium, follicular	hypertrophy	2	
A	30	29	A	epithelium, follicular	hypertrophy	2	
A	30	30	A	epithelium, follicular	hypertrophy	2	
A	30	27	A	thyroid gland	thyroglossal duct cyst	2	

Principle Investigator: A. Warren, Ph.D.

Table 2
Ammonium Perchlorate
Histopathology Database

Pathologist: J.R. Latendresse, D.V.M., Ph.D.
Diplomate, ACVP

Study ID	Dose mg/kg/day	Animal ID	Slide ID	Site	Diagnosis	Severity	Remarks
D	0	1	A	thyroid adventicia	ectopic thymus		
D	0	3	A	thyroid gland	essentially normal tissue		Follicles are variably sized. Follicular epithelium is low to medium cuboidal with the cytoplasmic to nuclear ratio usually equal to or less than 1. Cytoplasm is often vacuolated.
D	0	4	A	thyroid gland	essentially normal tissue		
D	0	5	A	thyroid gland	essentially normal tissue		
D	0	6	C	thyroid gland	essentially normal tissue		
D	0	2	A	thyroid gland	thyroglossal duct cyst	2	Incidental congenital cyst commonly formed postnatally due to accumulation of proteinaceous fluid in thyroglossal duct remnant.
D	30	25	C	thyroid follicle	colloid depletion	2	Follicles are predominantly small to medium with decreased luminal size and colloid.
D	30	26	A	thyroid follicle	colloid depletion	2	
D	30	27	A	thyroid follicle	colloid depletion	2	
D	30	28	A	thyroid follicle	colloid depletion	2	
D	30	29	C	thyroid follicle	colloid depletion	2	
D	30	25	C	capillary	congestion	2	Inter- and intrafollicular capillaries are prominantly, diffusely dilated and filled with erythrocytes.
D	30	26	A	capillary	congestion	2	
D	30	27	A	capillary	congestion	2	
D	30	28	A	capillary	congestion	2	
D	30	29	C	capillary	congestion	1	
D	30	25	C	epithelium, follicular	hypertrophy	2	Follicles are variably sized. Height of the follicular epiththem is usually high cuboidal to low columnar. Area of follicular cytoplasm is usually 1.5 to 2x greater than controls making cytoplamic to nuclear ratio about 1.5 to 2. Perinuclear halo often present.
D	30	26	A	epithelium, follicular	hypertrophy	2	
D	30	27	A	epithelium, follicular	hypertrophy	2	
D	30	28	A	epithelium, follicular	hypertrophy	2	
D	30	29	C	epithelium, follicular	hypertrophy	1	
D	30	30	C		NOT EXAMINED		RECUT, NOT ENOUGH TISSUE TO EVALUATE.

From: Latendresse, John <JLatendresse@nctr.fda.gov>
To: 'Alan Warren' <awarren@terra1.com>
Date: Monday, January 11, 1999 3:33 PM
Subject: RE: slides

Alan,
I didn't read your message until after I had sent the report out. With few exceptions, I have never been a strong advocate of "blind" histopathology assessment of toxicology studies. Blind reading generally takes much longer, and it can significantly hinder the identification and characterization of lesions induced by exposure to a xenobiotic agent, particularly when they are suttle. With such a study like ammonium perchlorate, I believe that one would get a much more accurate and confident characterization of morphologic alterations by first comparing the high dose and control specimens to establish thresholds for severity scores, for example. Particularly when lesions are suttle, this is an absolutely essential step precluding one's attempt to determine a dose response. To summarize, frankly, in most instances I believe you don't need a blind reading to get a quality, unbiased assessment by the majority of pathologists who characterize morphologic alterations for a living. Often such requests come from scientists who don't understand the process of morphologic assessment. Most pathologists worth their salt actually do some sort of a blind reading anyway, if the study implies a need. For example, after I have carefully compared the morphology of control and high dose specimens, and detect a putative morphologic alteration believed to be due to exposure to a toxicant, I will confirm my observation by examing a pool of unknown specimens. If I can separate the treatment and control specimens based on the morphologic criteria developed during the high dose and control comparison, I proceed with a similar series of exercises in an effort to define a dose response.